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Population Ecology of the Brook Trout, *Salvelinus fontinalis*, in Matamek Lake, Quebec[1]

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SAUNDERS, L. H., AND G. POWER. 1970. Population ecology of the brook trout, *Salvelinus fontinalis*, in Matamek Lake, Quebec. J. Fish. Res. Bd. Canada 27: 413–424.

Population estimates of an unexploited brook trout population at Matamek Lake, Quebec, indicated that more than 86% of the total population inhabited the tributaries of the lake during the summer of 1968. As opposed to the stream habitat, the combination stream–lake ecosystem moderated the mortality rates of the intermediate age-groups and allowed survival to higher ages in the population. Estimates of standing stock and production in the lake were 3.1 and 2.2 kg/ha respectively during 1968.

The population was characterized by a slower growth rate, higher age at maturity, higher survival rate in the intermediate age-groups, and longer life span when compared with an exploited population in Wisconsin. Increased numbers of trout in higher age-groups at Matamek Lake were attributed to high survival rates in the intermediate age-groups in the absence of exploitation. Low estimates of standing stock and production were attributed to environmental factors affecting growth.

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INTRODUCTION

THIS PAPER DESCRIBES the population ecology of brook trout, *Salvelinus fontinalis* (Mitchill) in Matamek Lake, Quebec. Although this species is widely distributed and abundant throughout the interior of Quebec, Labrador, and Newfoundland, it has been neither extensively exploited nor studied due to the relatively inaccessible nature of the terrain. In the more accessible reaches of its range the brook trout is a favorite of anglers, and many aspects of its biology have been studied in detail. Population ecology and production studies in the northern United States have dealt mainly with exploited populations in streams (McFadden, 1961; Hunt, 1966) or lakes (Hatch and Webster, 1961). Comparative studies are now being conducted on populations that are not seriously exploited by man (Cooper, 1967). Little research has been conducted on unexploited brook trout populations or on populations that inhabit a combination stream–lake ecosystem in Quebec. The Matamek Lake ecosystem provided a population in which these conditions were met and this study was designed to investigate the population ecology of the brook trout under these circumstances.

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DESCRIPTION OF THE LAKE

Matamek is a large, deep, oligotrophic lake located 35 km northeast of Sept-Iles, Quebec. It has an area of 1620 ha and a maximum depth of 100 m. The water is dark brown, the *p*H 5.8, and the total alkalinity 5 ppm ($CaCO_3$). During the summers of 1967–68 there was no indication of oxygen depletion at any depth. There is an obvious paucity of littoral area that is restricted to the exposed northern shore, shallow bays, and river entrances. The lake lies within the St. Lawrence forest region, and the substrate is predominately granite and gneiss.

The lake forms a natural reservoir in the Matamek River system (Fig. 1). The lake surface has an elevation of 122 m, and anadromous species are unable

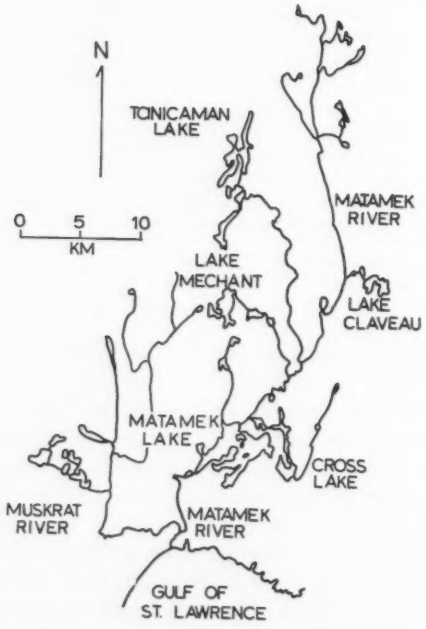

FIG. 1. The Matamek watershed, Saguenay County, Quebec.

to reach the lake from the Gulf of St. Lawrence at present. Brook trout are abundant in the shallow littoral areas of the lake. Large populations of American smelt, *Osmerus mordax*, and threespine stickleback, *Gasterosteus aculeatus*, are also present. Arctic char, *Salvelinus alpinus*, inhabit the deeper reaches of the lake in small numbers. The ninespine stickleback, *Pungitius pungitius*, is found in small numbers where running water enters the lake.

METHODS

A total of 1400 brook trout were collected during the summers of 1967–68 to provide data on age, growth, and fecundity. Fish were captured with overnight sets of gillnets (¾–4 inches

stretched mesh) throughout the lake. All specimens were measured (fork lengths), weighed, sexed, and assigned to an age-group.

Age and growth were determined from scale analysis and its relationship with fish length. Scale samples were removed from the left side between the lateral line and dorsal fin. These were mounted (Power, 1964) and each fish was assigned to an age-group after scale analysis (Cooper, 1951). The mean lengths of males and females in the various age-groups were calculated, and the average increase in mean length between age-groups was used as a measure of annual growth of the lake population. An age–length key (Table 1) was prepared from the scale analyses for use in the population estimates (Allen, 1966).

TABLE 1. Age–length key prepared from scale analyses of trout, Matamek Lake, 1967–68.

Fork length (*mm*)	Age							
	0+	1+	2+	3+	4+	5+	6+	7+
40–59	4							
60–79	5	8						
80–99		24	9					
100–119			57					
120–139			85	12				
140–159			7	79	1			
160–179			1	354	10			
180–199				173	82	1		
200–219				7	175	4		
220–239				2	115	18		
240–259					16	61	3	
260–279						28	10	1
280–299						5	11	2
300–319						3	8	2
320–339						1	7	4
340–359							1	2
360–379								1
380–399								
400–419								1
Total	9	32	159	627	399	121	40	13
%	1.0	2.3	11.4	44.8	28.5	8.6	2.8	2.1

Sex ratios, age at maturity, and fecundity were determined from gross examinations of gonads. Sexual maturity was recorded using a scale from 0 to 5, and all females in categories 0–2 (eggs less than 2 mm diam) were considered nonspawners in 1968. Ripe ovaries (eggs greater than 2 mm diam) were removed and later analyzed for fecundity using the method of Vladykov (1956). Average numbers of eggs per female and numbers of spawning females were calculated for the various age-groups and later used to estimate egg production in the lake.

Population estimates for the lake were based on an extensive mark-and-recapture procedure during the summer of 1968. Trout were captured for marking by seining, trap netting, and angling. Only yearling and older trout were included in the lake estimates. Fry were observed at the entrances of tributaries into the lake, but they occurred only in small numbers and were considered more closely associated with the streams than with the lake. A total of 3555 trout were anesthetised with M.S. 222 and marked (Kelly, 1967) with virtually no handling mortality. The initial capture methods plus gillnets (¾–4 inches) were employed to recapture marked fish. This combination of capture methods was chosen to nullify some of the gear selectivity that may

occur when a single capture method is employed. Data on the numbers of marked trout recaptured in relation to numbers marked and totals captured in the various length categories (Table 2) were accumulated on a daily basis for a Schnabel (1938) estimate. Estimates of the numbers of fish in the various age-groups (Table 3) were calculated from the length-frequency distribution (Table 2) and the age–length key (Table 1).

Population estimates of the numbers of trout residing in the nursery streams were calculated from their annual survival rates and the estimate of the most abundant age-group in the lake. Single rotenone samples were taken in three major tributaries during the summer of 1968. Ages

TABLE 2. Length-frequency distribution of marked trout recaptured in relation to numbers marked and totals captured in the various length categories, Matamek Lake, 1968.

Fork length (*mm*)	Total captured	No. recaptured	No. marked
60–79	24	1	7
80–99	126	16	52
100–119	503	109	273
120–139	785	162	597
140–159	1065	157	817
160–179	765	122	717
180–199	450	94	448
200–219	299	53	331
220–239	146	21	162
240–259	79	16	95
260–279	21	2	35
280–299	13	3	8
300–319	5	–	8
320–339	4	1	3
340–359	1	–	2
Total	4287	757	3555

TABLE 3. Schnabel estimates of numbers of fish in various age-groups, Matamek Lake, 1968. Calculated from length-frequency data (Table 2) and age–length key (Table 1).

Age	Total captured	No. recaptured	No. marked	Schnabel estimate
1+	35	3	36	1,426
2+	1,108	227	1,033	20,359
3+	2,116	344	1,660	38,627
4+	835	151	669	14,303
5+	162	28	135	2,918
6+	31	4	22	487
Total	4,287	757	3,555	78,120±10,468[a]

[a]Confidence limits, equal to two standard errors, calculated from the formula:

$$\text{SE}(\hat{N}) = \hat{N}\sqrt{\frac{(\hat{N}-m)(\hat{N}-c)}{mc(\hat{N}-1)}}$$

where N = population estimate, m = total number of marked fish in the population, c = number of fish in the sample (Robson and Regier, 1968).

were assigned by scale analyses, and the annual mortality rates (Regier and Robson, 1967) were calculated for the various age-groups. The number of age 2+ trout required to yield the number of age 3+ trout in the lake was estimated using the formula $n = \frac{x}{y}$ where n = the number of age 2+ trout required, x = the number of age 3+ trout present, and y = the annual survival of age 2+ trout in the tributaries. Since the value of n represented the total number of age 2+ trout required, the estimated number of age 2+ trout in the lake was subtracted to obtain the number of age 2+ trout in the streams. From this estimate the numbers of trout in the various age-groups in the tributaries were calculated using their annual survival rates.

This method of population estimation was employed with the assumption that reproduction was sufficiently uniform throughout the year-classes represented in the population to allow accurate determinations of mortality. Boccardy and Cooper (1963) have demonstrated that brook trout 2–5 inches long are approximately equal in susceptibility to capture by rotenone in small streams. Some of the fry at Matamek did not reach this minimum in their first year and may not be adequately represented in the samples. Consequently, the mortality figures for age 0+ trout should be considered minimal. The amount of error inherent in the stream sampling was not calculated, but its absence was not considered sufficient reason to reject the generalizations based on the subsequent population estimates.

The population estimates for the lake and the streams were combined to provide the total population estimate that was used to calculate annual mortality, total annual mortality (Jackson, 1939), standing stock, and production. Annual mortality was divided into natural death and emigration in the streams. Mortality due to emigration was equated to the differences in mortality between the total population and the stream population, and the numbers of trout migrating to the lake in the various age-groups were estimated from these rates. The standing stock was estimated by summing the products of the population estimates and mean weights for the various ages. Production, kilograms of body tissue elaborated per year, was estimated using the graphical method of Allen (1951). Production was not estimated for fry, for mortality in the first year of life has been proven too variable to be represented by an average rate (Latta, 1962). Production by age 0+ trout was taken as the standing stock and represented an underestimate. Both standing stock and production were estimated independently for lake and stream populations due to differences in growth rates. Most empirical calculations were performed by the IBM 360/75 computer (Fortran IV) using programs developed by Allen (1966), Dahlberg (1966), and Sheri (MS, 1968).

RESULTS

Population Estimates and Distribution

Population estimates indicated that more than 86% of the total population resided in the streams during the summer (Table 4). The lake population was composed primarily of age 2+ and older trout. Trout attained a maximum of age 4+ in the streams and age 7+ in the lake. As opposed to the stream habitat, the combination stream–lake ecosystem altered the age-group structure and allowed an increase in the life span of the total population.

Mortality

Annual mortality rates were dissimilar for the various age-groups in the streams, lake, and total population (Table 5). Mortality rates for the streams were:

Age	0+	1+	2+	3+	4+
Sample size	260	107	83	30	7
Annual mortality	.59	.22	.64	.77	

TABLE 4. Estimates of numbers of trout and annual mortalities at various ages in the streams and the lake, 1968.

	Tributaries		Lake		Total	
Age	No. of fish	Annual mortality	No. of fish	Annual mortality	No. of fish	Annual mortality
Egg	4,374,975				4,374,975	
		.94				.94
0+	271,859				271,859	
		.59				.58
1+	111,462		1,426		112,888	
		.22				.05
2+	86,941		20,359		107,300	
		.64				.35
3+	31,300		38,627		69,927	
		.77		.63		.69
4+	7,200		14,303		21,503	
				.80		.86
5+			2,918		2,918	
				.83		.83
6+			487		487	
Total	508,762	.53	78,120	.68	586,882	.56

TABLE 5. Estimates of annual mortality due to death and emigration in the trout population, 1968. In parentheses are numbers of trout migrating to Matamek Lake.

	Streams			Lake	Total
Age	Natural death	Emigration	Total	Natural death	Natural death
Egg					
	.94		.94		.94
0+					
	.58	.01 (2,718)	.59		.58
1+					
	.05	.17 (18,948)	.22		.05
2+					
	.35	.29 (25,213)	.64		.35
3+					
	.69	.08 (2,504)	.77	.63	.79
4+					
				.80	.86
5+					
				.83	.83
6+					
Total			.53	.68	.56

Mortality rates for the total population were greatest during the first and last 2 years of life with the intermediate age-groups having moderate mortality rates. The greatest number of trout migrated to the lake between age 1+ and age 3+ (Table 5), and the decrease in mortality in these age-groups was attributed to a decrease in population pressure through emigration. This may also be explained by postulating a weak year-class of age 1+ trout, but this could only be clarified by more sampling through several years.

Growth

Comparisons of the mean lengths of males and females in various age-groups suggested that the differences observed were minimal (Table 6). For

TABLE 6. Mean lengths of males and females at various ages, Matamek Lake, 1967–68.

	Males					Females				
Age	No. in sample	Mean length (*mm*)	Range	SD of mean	SE of mean	No. in sample	Mean length (*mm*)	Range	SD of mean	SE of mean
1+	4	86	82–92	4.2	2.1	1	96	96–96		
2+	56	125	93–154	10.5	1.4	54	122	92–170	13.3	1.8
3+	259	172	130–230	12.9	.8	340	172	121–225	14.2	.7
4+	256	215	179–258	15.2	.9	142	207	155–245	17.2	1.4
5+	95	252	204–334	20.4	2.1	32	248	197–304	19.4	3.5
6+	31	294	247–343	26.5	4.6	9	295	250–323	19.9	6.6
7+	11	338	276–415	45.4	13.7	2	331	327–335	5.6	4.0

the combined sexes the annual increments in length and the percentage increases for the various age-groups were:

Age	0+	1+	2+	3+	4+	5+	6+
Lake (*mm*)	37.6	37.7	50.6	39.8	39.7	42.4	40.0
(%)	81.7	45.1	41.7	23.6	18.8	16.9	13.6
Streams (*mm*)	32.5	38.7	49.9	23.9			
(%)	69.7	48.9	42.2	14.3			

In the streams and the lake growth was fairly uniform during the first 2 years. In both habitats the absolute growth was greater at age 2+ than at other ages, and this increase was attributed to decreased population pressure in the streams and increased food supply resulting from migration to the lake. The differences in absolute growth at age 3+ for the lake and streams suggested that the lake habitat offered advantages favorable to more rapid growth and increased survival that were not available in the streams.

Age at Maturity, Sex Ratios, and Fecundity

In the lake both sexes matured at nearly equal rates, and by age 3+ more than 95% of the population was sexually mature (Table 7). Males were

TABLE 7. Percentages of males and females mature at various ages and sex ratios, Matamek Lake, 1967–68.

Age	Males		Females		Sex ratios M:F
	No. in sample	% mature	No. in sample	% mature	
1+	26	15	6	17	80:20
2+	82	68	80	68	50:50
3+	272	95	356	96	46:54[a]
4+	261	98	144	99	64:36
5+	95	100	32	100	75:25
6+	31	100	9	100	78:22
7+	11	100	2	100	85:15
Total	778	93	629	92	55:45

[a]Probably closer to 50:50 ratio because mature females were selected, not randomly sampled, in September for fecundity data.

more numerous than females in age-groups greater than age 3+ and in the total population. The mean number of ova per spawning female increased with age (Table 8). Estimates of the numbers of spawning females and egg production for the lake in 1968 were:

Age	2+	3+	4+	5+	6+	Total
Estimated no. of females	2,215	15,018	4,690	730	730	22,760
No. of ova	143,975	2,628,150	1,292,850	292,000	68,480	4,374,975
% of total	3.3	60.0	28.5	6.7	1.6	100.0

More than 88% of the total egg production was contributed by age 3+ and age 4+ females. Although age-groups 5+ and 6+ produced the largest number of eggs per individual, they contributed relatively little to the total production.

TABLE 8. Lengths, weights, and numbers of ova for females of various ages, Matamek Lake, 1968.

Age (approx)	No. in sample	Mean length (*mm*)	Mean weight (g)	Mean no. of ova per fish	Egg diam (*mm*)
2+	6	212	20	55	2.88
3+	51	174	62	165	3.33
4+	32	212	109	292	3.68
5+	16	245	170	369	4.02
6+, 7+	13	355	536	1203	3.88

STANDING STOCK AND PRODUCTION

The amounts of biomass present in the two habitats were nearly equal, and most of the total standing stock was contributed by the intermediate

age-groups (Table 9). Standing stock was estimated to be 3.1 kg/ha (2.8 lb/acre) in the lake, but the areas of the streams were not determined and estimates per unit area were not made. Production was substantially greater

TABLE 9. Distribution of biomass (kg) in the trout population, 1968.

	Stream			Lake			Total	
Age	Estimated no.	Mean wt (g)	Standing stock (*kg*)	Estimated no.	Mean wt (g)	Standing stock (*kg*)	Estimated no.	Standing stock (*kg*)
0+	271,859	2.0	543.7				271,859	543.7
1+	111,462	6.5	724.5	1,426	8.1	11.5	112,888	736.0
2+	86,941	21.4	1,860.5	20,359	21.7	441.4	107,300	2,301.9
3+	31,300	52.6	1,645.1	38,627	58.9	2,273.6	69,927	3,918.7
4+	7,200	80.8	581.4	14,303	110.4	1,579.2	21,503	2,160.6
5+				2,918	188.2	549.3	2,918	549.3
6+				487	309.4	150.7	487	150.7
Total	508,762		5,355.2	78,120		5,005.7	586,882	10,360.9
%	86.6		51.6	13.4		48.4	100.0	100.0

in the streams than in the lake and this may be attributed to the large numbers and rapid growth of young fish (Table 10). Production per individual was greater in the lake than in the streams and probably reflected decreased main-

TABLE 10. Production (kg/year) of the trout population, 1968.

	Stream			Lake			Total	
Age	Estimated no.	Growth (*g/year*)	Production	Estimated no.	Growth (*g/year*)	Production	Estimated no.	Production
0+	271,859		543.7				271,859	543.7
		4.5						
1+	111,462		862.5	1,426		11.5	112,888	874.0
		14.9			13.6			
2+	86,941		1,661.9	20,359		148.2	107,300	1,810.1
		31.2			37.2			
3+	31,300		1,844.6	38,627		1,097.2	69,927	2,941.8
		28.2			51.5			
4+	7,200		542.8	14,303		1,362.9	21,503	1,995.7
			90.0		77.8			
5+				2,918		666.4	2,918	666.4
					121.2			
6+				487		206.3	487	250.4
						44.1		
Total	508,762		5,545.5	78,120		3,536.6	586,882	9,082.1
%	86.6		61.1	13.4		38.9	100.0	100.0

tenance requirements (no current) and increased food value (more prey species). Again, it was apparent that the intermediate age-groups contributed most of the annual production of the total population. Production in the lake was estimated as 2.2 kg/ha (1.9 lb/acre). Total production and standing stock were almost equal in 1968 suggesting one complete turnover in biomass per year.

DISCUSSION

The total population occupied an ecosystem composed of the lake and stream habitats at Matamek. As opposed to the stream habitats alone, the stream–lake ecosystem permitted a larger population size, sustained growth beyond age 3+, higher survival rates of the intermediate age-groups, and increased longevity of individuals.

The Matamek population had a longer life span, slower growth rate, and higher age at maturity than the Lawrence Creek population (McFadden, 1961). The maximum ages attained in the two populations were different, but the maximum sizes attained were similar (Fig. 2). The ages at which 95% of the

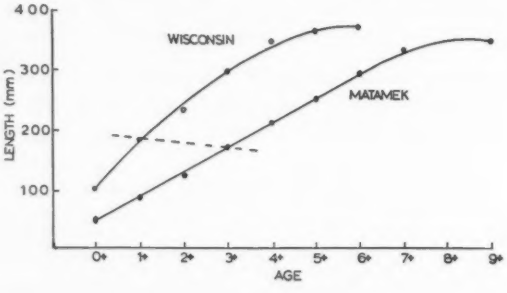

FIG. 2. Growth rates and mean ages at maturity (broken line) in brook trout from Lawrence Creek, Wisconsin, and Matamek Lake.

two populations reached sexual maturity were different, but the lengths at which this occurred were similar suggesting that maturity was more closely associated with size than age. The values obtained for fecundity in the lake (Table 8) were within the ranges cited for brook trout in Quebec (Vladykov, 1956; Power, 1966), but lower than those reported from Lawrence Creek. Graphical comparison of fecundity in Matamek Lake and Lawrence Creek (Fig. 3) suggested that it differed in the same manner as growth (Fig. 2). The differences in these parameters between the two populations appeared to be the result of slower-growing fish reaching sexual maturity at a higher age and producing fewer eggs than faster-growing fish. Lower temperatures and shorter growing seasons resulting in a low metabolic turnover of nutrients, slow growth rate, and long life span (Carlander, 1966) may be the explanation of these differences.

The age-group structure of the Matamek population was characterized by the presence of many individuals in the higher age-groups. This contrasted with the Lawrence Creek population in which trout older than age 2+ were

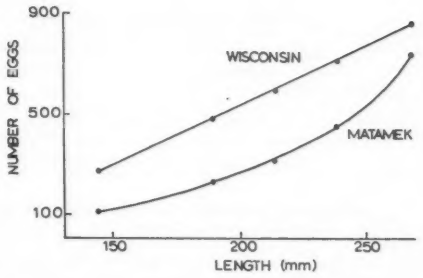

FIG. 3. Fecundity of brook trout from Lawrence Creek, Wisconsin, and Matamek Lake.

rarely encountered (Fig. 4). The differences in age-group structure in the two populations were attributed to high survival rates in the intermediate age-groups resulting in increased numbers of trout in higher age-groups in the absence of exploitation. The Matamek data are more likely to represent a population in equilibrium with its natural environment, whereas comparison of the two populations may demonstrate the effects of exploitation on natural populations.

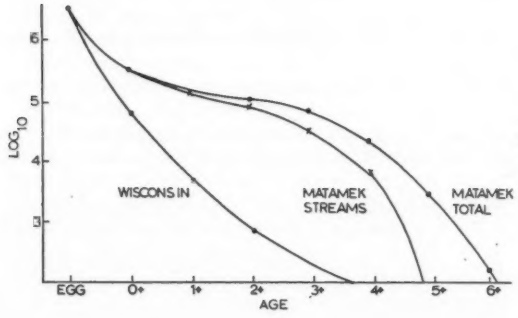

FIG. 4. Numbers of trout in the various age-groups, Lawrence Creek, Matamek streams, and Matamek total populations.

Standing stock and production were low at Matamek Lake when compared with other studies. A mean standing stock of 4.1 lb/acre has been reported for 18 trout lakes from Wyoming to Nova Scotia (Carlander, 1955). New York mountain lakes averaged 6–12 lb/acre annual production (Hatch, 1961). The low standing stock may be the result of depth, alkalinity, and the presence of closely related species (Carlander, 1955) at Matamek Lake. Low annual production may be attributed to the slow growth of trout in the lake.

ACKNOWLEDGMENTS

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Effects of Acid Mine Wastes on Phytoplankton Communities of Two Northern Ontario Lakes

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JOHNSON, M. G., M. F. P. MICHALSKI, AND A. E. CHRISTIE. 1970. Effects of acid mine wastes on phytoplankton communities of two northern Ontario lakes. J. Fish. Res. Bd. Canada 27: 425–444.

Differences in chemical composition in 1965–67 between two lakes contaminated by uranium-milling wastes and one unaffected lake were related directly to the nature of processes in uranium extraction and subsequent waste treatment. Low *p*H and high concentrations of SO_4^{-2}, NO_3^{-1}, and Ca^{+2} occurred in the contaminated lakes. Low concentrations of inorganic carbon, which apparently limited production in the contaminated waters, resulted from reduced solubility, loss, and possibly inefficient regeneration of CO_2. Other major nutrients did not appear to be limiting, since nitrates were in greater supply in the contaminated lakes than in the unaffected lake and phosphorus and silica occurred in similar concentrations in all three lakes.

Lower phytoplankton populations and lower indices of diversity were found in the contaminated lakes than in the unaffected one. Many species of Bacillariophyceae, Chrysophyceae, and Myxophyceae developed in the unaffected lake but were absent or occurred in extremely low numbers in the contaminated lakes.

Average primary productivities were approximately 70 and 30 mg C m^{-2} day^{-1} in the two contaminated lakes and 130 mg C m^{-2} day^{-1} in the unaffected lake. In situ areal and volumetric measurements in laboratory and field bioassays confirmed the importance of inorganic carbon in limiting primary productivity. A potential compensatory mechanism in the contaminated lakes was a deepening of the euphotic zone. Although greater concentrations of inorganic carbon occurred and were assimilated in hypolimnetic than in epilimnetic waters, the mechanism was not sufficient to overcome the effects of reduction in both species diversity and abundance of phytoplankton on the areal primary productivity.

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INTRODUCTION

LARGE DEPOSITS of uranium-bearing ore were discovered in 1953 in the Serpent River watershed on the north shore of Lake Huron. By the spring of 1958 approximately 35,000 tons of ore were being processed daily by acid leaching in 10 mills. One ton of solids and 2–5 tons of waste water are disposed of per ton of ore milled. Small lakes and dyked swampy depressions are used to contain the solid wastes. The decant from these tailings areas flows to the large lakes of the Serpent River system.

The present study on phytoplankton communities and primary productivity was carried out in 1965–67 on two contaminated lakes and one unaffected lake. Although production of uranium had decreased by 1965, marked changes in the chemical and physical properties of the affected lakes had already occurred, of which the decrease in *p*H appeared to be the most serious. Levels

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of radioactivity in lake waters, particularly of ^{226}Ra, were of public health significance (Province of Ontario, 1965). However, the increase over background levels to about 10-fold in the most affected lakes, although possibly significant, may be considered less important than gross chemical alterations affecting phytoplankton communities.

This report considers: differences in general physicochemical limnology between contaminated and unaffected lakes during the period 1965–67, comparison of the specific compositions and distributions of the phytoplankton in 1966–67, and comparison of primary productivities in 1965–66. We will attempt to indicate the manner in which excess, free-mineral acidity in mine wastes promotes the depletion of inorganic carbon and leads to virtual elimination of important components of the flora, which together affect primary productivity.

The Serpent River rises north of Dunlop Lake and flows from Dunlop Lake to Quirke Lake, where considerable mill waste water is received (Fig. 1). Thence it flows east and south through several other lakes, including Pecors Lake, to the North Channel of Lake Huron. The watershed consists of outwash sands interspersed with outcrops of precambrian igneous and metamorphic rock.

Dunlop Lake is 2020 acres (820 ha) in area and 38 m deep. No mill wastes have entered Dunlop Lake, and it is typical of uncontaminated lakes in the precambrian Shield of Northern Ontario (Ryder, 1964).

Quirke Lake has an area of 4600 acres (1860 ha) and maximum depth of 100 m. Six mills on Quirke Lake processed approximately 20,000 tons of ore daily and much of the waste water from nearby disposal areas entered the lake. Comparison of Quirke Lake with Dunlop Lake is logical because of its proximity and the fact that Dunlop Lake feeds Quirke Lake. However, the morphometry of Quirke Lake indicates the possibility of sparser phytoplankton under natural conditions.

Pecors Lake, the second contaminated lake, was studied because it is morphometrically similar to the eastern basin of Dunlop Lake. It is 720 acres (290 ha) in area and 40 m deep. In contrast with Quirke Lake, Pecors Lake receives flows displaced from contaminated lakes. Because the limnology of both contaminated lakes is influenced by characteristics of their feeding lakes, Pecors Lake might be expected to be more susceptible to damage from acid mine wastes than Quirke Lake. The latter might be benefited by waters received from uncontaminated Dunlop Lake.

METHODS

Physical and Chemical

Temperature readings with a telethermometer were made at the times of algal sampling. Penetration of light was determined with a Secchi disc in 1965 and with a Gemware submarine photometer in 1966.

Water samples from the three lakes were taken to the laboratory for chemical analyses. We examined the possibility that concentrations of heavy metals might have increased in the con-

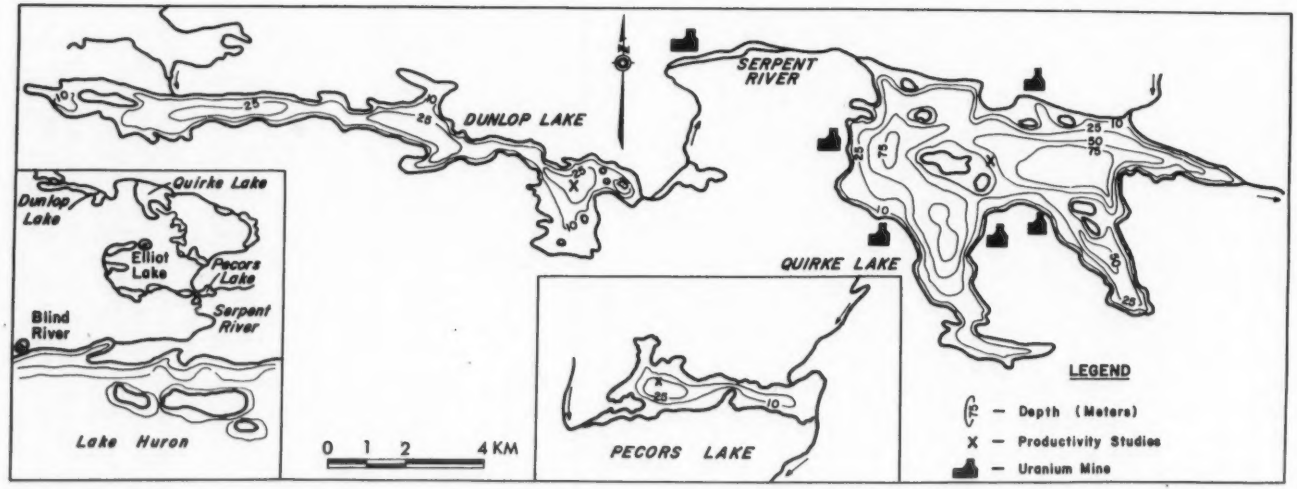

FIG. 1. Locations of the study area on the north shore of Lake Huron (inset), sampling stations, and uranium mills and depths of the three lakes examined.

taminated lakes. All analyses were conducted following standard procedures (American Public Health Association, 1965).

Collection and Examination of Algae

Phytoplankton samples were collected at a centrally located station (Fig. 1) at six depths from the surface to 30 m in Quirke Lake and to 23 m in the eastern basin of Dunlop Lake and in Pecors Lake. In 1965, samples were collected from Quirke and Dunlop lakes at the three times when productivity was measured. In 1966, samples were taken twice-monthly from March 31 through November 15 from Dunlop and Quirke lakes and on 3 days (June 29, August 17, and September 28) from Pecors Lake. In 1967, twice-monthly samples were collected from the three lakes between May 16 and October 2. All samples were preserved with mercuric chloride at the time of sampling.

Because of the unproductive nature of the lakes, all samples were concentrated using the Sedgwick-Rafter sand-filtration technique (American Public Health Association, 1965). The use of sand together with silk bolting cloth with apertures of 25 μ apparently retained most of the nannoplankton, although a few small forms probably were lost. Compositions of standing stocks of phytoplankton were determined as numbers of organisms per milliliter with the Sedgwick-Rafter counting cell and a magnification of 200×. Also, standing stocks of phytoplankton were measured in areal standard units (asu) for comparison with estimates of primary productivity (one asu is equal to an area of 400 μ^2). The areal unit was employed because it is as useful as cell volume and is preferred over cell numbers when relating primary productivity to standing stocks (Paasche, 1960).

Species composition was determined by examining permanent and semipermanent slide mounts at 1200×. Identification of the Bacillariophyceae to the species level was facilitated by acid digestion of the samples, followed by mounting in either Hyrax or Mikrops media. Permanent mounts of other forms were prepared with glycerin jelly as outlined by Pennak (1953). Taxonomic references included those of Prescott (1951), Patrick and Reimer (1966), Tiffany and Britton (1952), Smith (1950), and Sieminska (1964).

Diversity in Phytoplankton Communities

The index of diversity, I (after Margalef, 1958), for each depth on each sampling date for the three lakes was calculated as

$$I = \frac{S - 1}{\log_e N}$$

where S is the total number of species and N is the total number of individuals.

Margalef's I is assumed to be independent of the numbers of individuals examined. Development of I using cumulative counts of algae indicated that this assumption was correct, provided that at least 50 individuals were examined. Between 50 and 375 individuals were examined in each sample.

Primary Productivity

Estimates of primary productivity were made concurrently in Dunlop and Quirke lakes by the ^{14}C method on 3 days in 1965 (June 17, August 10, and October 2) and on 3 days in 1966 (June 14, August 13, and September 28). Estimates in Pecors Lake were made on June 29, August 17, and September 29, 1966; these days had weather conditions similar to those that prevailed when the other two lakes were examined previously.

Samples of lake water were collected at dawn in Van Dorn bottles from six depths at equal intervals from 1 to 30 m in Quirke Lake and from 1 to 23 m in Dunlop and Pecors lakes. In 1965, deeper samples taken from the former two lakes were found to be below the compensation depth. Water from each depth was dispensed into two clear and two dark (opaque) glass-stoppered bottles. $NaH^{14}CO_3$ (New England Nuclear Corp.; 100 μg in 1.0 ml with activity of 10 μCi) was added to each. The bottles were suspended for 6–9 hr at the depth from which the water was collected. Activity was arrested by the addition of mercuric chloride when the samples were

removed and, within several hours, the algae were removed on 0.45-μ Millipore filters by use of moderate pressure (75 cm Hg). Each filter was rinsed with distilled water, dried, and placed in scintillation-counting medium of 0.01% POPOP (1,4-bis-2-(5-phenyloxazolyl)-benzene) and 0.4% PPO (2,5-diphenyloxazole) in toluene. The ^{14}C-activity of algae was determined with a Packard Tricarb spectrometer.

The use of mercuric chloride was investigated by comparing the activity of preserved algae with that of other subsamples filtered at the end of incubation. Although the preserved algae usually had slightly lower specific activity, the difference was within the limits of precision of the overall method.

The possibility of underestimating assimilation of ^{14}C, because of the loss by excretion of assimilated activity, was investigated. Filtrates were saved from 16 field analyses of ^{14}C assimilation, representing both light and dark samples from the epilimnion and hypolimnion of Quirke and Dunlop lakes. No evidence of significant excretion of assimilated ^{14}C was obtained when subsamples of filtrates were acidified, stripped of inorganic carbon by bubbling with nitrogen, and their residual ^{14}C activities (organic carbon) determined by scintillation counting in dioxane-napthalene with PPO and POPOP.

Calculation of inorganic carbon based on *p*H and direct titration of alkalinity was not advisable because of the small amount present. Therefore, duplicate analyses were carried out by distillation of inorganic carbon at low *p*H into 0.02 N NaOH in a closed, circulating system. The base was titrated with 0.05 N HCl. The titre was corrected with "blank" determinations on samples of freshly boiled, distilled water. Because this procedure led to delay in analysis, samples were preserved immediately after collection with mercuric chloride and the *p*H was adjusted to 8–9 with barium hydroxide.

The uptake of carbon by phytoplankton was calculated as

$$1.05 \frac{Y}{Z} \cdot (W + X) \text{ mg C m}^{-3}$$

where 1.05 corrects for isotopic discrimination (Strickland, 1960), Z is activity of inorganic carbon added, Y is activity of filtered phytoplankton corrected for dark-bottle assimilation, W is inorganic carbon concentration in lake water (mg C m^{-3}), and X is concentration of inorganic carbon added to bottles.

Duplicate analyses of productivity based on independent measurements of light-bottle and dark-bottle activities and inorganic carbon showed a coefficient of variation of 20% in data from Quirke and Dunlop lakes. Greater relative variation in analyses of inorganic carbon at the low concentrations in Quirke Lake (1 mg C $liter^{-1}$ and less) compared with Dunlop Lake (2 mg C $liter^{-1}$) caused generally poorer precision in estimating primary productivity in the former. Precision in ^{14}C counting was similar in the two lakes. Estimates of productivity in Pecors Lake should be within similar limits.

The hourly rate of carbon assimilation on an areal basis was integrated graphically. Daily assimilation was based on the proportion of the day's insolation received during the incubation period. There is some difference of opinion on whether productivity follows the daily insolation or is more efficient before midday. Goldman (1968) gave results that supported the former reponse, but several authors (Doty and Oguri, 1957; Ohle, 1958; and Vollenweider and Nauwerck, 1961) demonstrated the occurrence of asymmetrical day-curves. However, any bias introduced probably would affect the estimates of daily productivity similarly among the lakes.

Bioassays

Bioassays of possible limiting nutrients were conducted in early August 1966 in the laboratory in 190-ml bottles and in Quirke and Dunlop lakes in large polyethylene bags. The responses of phytoplankton to increased inorganic carbon and phosphate and to the two nutrients together were examined.

The polyethylene bags set in the lakes were 3.2 m long and contained 1 m^3 of water when set at the surface. One received 6 mg C $liter^{-1}$ as $NaHCO_3$ (which raised the *p*H of water from Quirke Lake to 6.7 but did not change significantly the *p*H of water from Dunlop Lake). The

second received 0.2 mg PO_4 liter^{-1} as $Na_2HPO_4 \cdot 12H_2O$, the third received both nutrients, and a fourth bag served as a control. After several hours, samples were drawn from a depth of 1.6 m in each bag and estimates of primary productivity were made in duplicate light bottles.

Duplicate samples from Quirke and Dunlop lakes were incubated in the laboratory at approximately 1 klux for 5 hr with ^{14}C and additions of 0.0, 1.5, 3.0, 6.0, and 9.0 mg C liter^{-1} with and without 0.2 mg PO_4 liter^{-1}. As in the field experiments, $NaHCO_3$ and $Na_2HPO_4 \cdot 12H_2O$ were used.

PHYSICAL AND CHEMICAL CHARACTERISTICS

Ionic Properties

Ionic composition of Dunlop Lake differed markedly from that of Quirke and Pecors lakes (Table 1). Concentrations of calcium, magnesium, sodium,

TABLE 1. Concentrations (mg liter^{-1}) of major ionic constituents and total dissolved solids in samples from Dunlop and Quirke lakes in 1965, 1966, and 1967 and from Pecors Lake in 1966 and 1967. All ions were measured directly by analysis except HCO_3^{-1}, which was calculated from total inorganic carbon and *p*H using the molecular proportions of total free CO_2 and HCO_3^{-1} (Hutchinson, 1957).

Cation or anion	Dunlop L.			Quirke L.			Pecors L.		
	Mean	Range	No. analyses	Mean	Range	No. analyses	Mean	Range	No. analyses
Ca^{+2}	4.1	2.0–7.8	86	38.1	34.0–54.0	85	66.5	38.0–91.1	52
Mg^{+2}	5.0	0.0–10.0	13	5.4	2.0–9.0	13	6.6	3.0–14.0	6
Na^{+1}	0.5	0.2–0.9	14	5.1	3.9–6.9	14	6.8	6.0–8.0	6
K^{+1}	0.5	0.3–0.9	14	5.3	4.2–6.1	13	8.6	7.0–11.0	66
SO_4^{-2}	7.1	0.0–26.1	66	105.8	72.0–221.0	83	163.4	92.0–252.0	45
Cl^{-1}	3.1	1.0–12.0	44	6.9	5.1–15.0	42	10.7	7.1–19.0	24
HCO_3^{-1}	6.5	2.5–11.5	72	0.5	0.1–1.3	72	2.3	0.4–3.6	30
NO_3^{-1}	0.4	0.0–2.0	73	11.6	2.8–22.0	80	12.8	4.4–30.0	48
Total dissolved solids	23.5	2.0–72.0	82	197.4	73.1–410.0	88	339.0	99.0–499.1	48

potassium, sulphate, chloride, and nitrate generally increased from Dunlop Lake to Pecors Lake. Most of this increase was composed of SO_4^{-2} and Ca^{+2}.

Concentrations of soluble iron (0.03 mg liter^{-1}), copper (0.02 mg liter^{-1}), zinc (0.05 mg liter^{-1}), and lead (0.04 mg liter^{-1}) in Quirke Lake, although greater than in Dunlop Lake, did not appear to be unusually high.

Inorganic Carbon and *p*H

Lakes of the Serpent River system are naturally low in inorganic carbon as shown by Dunlop Lake (Fig. 2), which showed a progressive depletion of inorganic carbon in the epilimnion and regeneration in the hypolimnion during the summer. In Quirke Lake the concentrations of inorganic carbon were lower than in Dunlop Lake and were erratically distributed with depth, although the surface concentrations were usually extremely low (Fig. 2). The

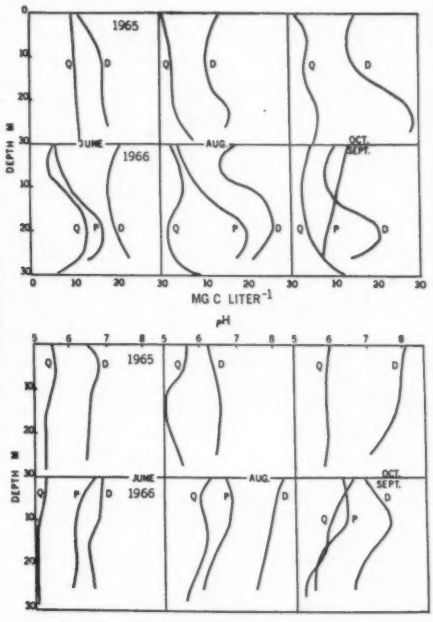

FIG. 2. Vertical variations in concentration of inorganic carbon and *p*H determined at the same times that primary productivity was measured. Samples were taken at six equidistant depths in each of the three lakes: Quirke Lake, Q; Pecors Lake, P; and Dunlop Lake, D.

few analyses of inorganic carbon from beneath the euphotic zone in Quirke Lake suggested that the lower hypolimnion was as poorly supplied as the upper. However, this was to be expected in a lake of such great depth, as was the orthograde oxygen distribution regularly observed in Quirke Lake (Ontario Water Resources Commission, unpublished data). Pecors Lake, which is morphometrically comparable to the eastern basin of Dunlop Lake, had concentrations of inorganic carbon intermediate between those of Quirke Lake and Dunlop Lake (Fig. 2).

The *p*H of Quirke Lake was less at every depth and on every occasion than that of Dunlop Lake (Fig. 2). Pecors Lake showed intermediate *p*H.

PHOSPHORUS, NITROGEN, AND SILICA

Concentrations of soluble and total phosphorus (Table 2) in the three lakes were similar, whereas nitrate and Kjeldahl nitrogen increased in order from Dunlop to Quirke to Pecors lakes. Compared with Dunlop Lake, slightly less orthosilicate was detected in Quirke and Pecors lakes.

LIGHT AND TEMPERATURE

Transparency was generally greater in the contaminated lakes than in the uncontaminated one (Fig. 3, 4). As measured by the Secchi disc in 1965 (Fig. 4), transparency was greater in Quirke Lake (15 m) than in Dunlop

TABLE 2. Nutrient concentrations (mg liter^{-1}) in Dunlop and Quirke lakes, 1965 through 1967, and in Pecors Lake, 1966 and 1967. Values of phosphorus are expressed as PO_4, total Kjeldahl nitrogen (including ammonia) and nitrate as N, and orthosilicate as SiO_2.

Nutrient	Dunlop L.			Quirke L.			Pecors L.		
	Mean	Range	No. analyses	Mean	Range	No. analyses	Mean	Range	No. analyses
Soluble phosphorus	0.03	0.00–0.21	58	0.03	0.00–0.32	64	0.05	0.00–0.28	46
Total phosphorus	0.07	0.00–0.70	91	0.06	0.00–1.40	95	0.06	0.00–0.56	44
Nitrate	0.10	0.00–0.50	73	2.90	0.70–5.50	80	3.2	1.10–7.50	48
Total Kjeldahl	0.69	0.07–2.50	43	1.80	1.00–8.30	87	2.36	0.26–9.90	57
Orthosilicate	0.89	0.22–2.10	41	0.54	0.24–0.90	22	0.58	0.28–0.79	14

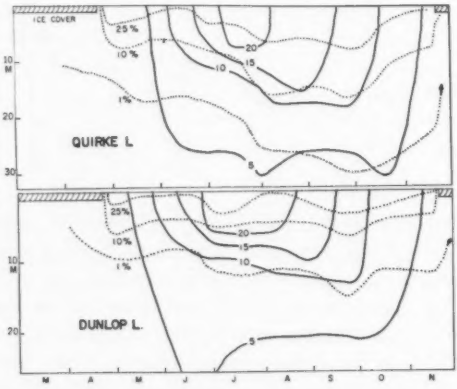

FIG. 3. Isotherms (solid lines) and isophots (dotted lines) in Quirke and Dunlop lakes determined by twice-monthly sampling in 1966.

Lake (8 m) in August, although it was 10 m for both lakes in June and October. The photometer measurements in 1966 showed similar transparencies in Quirke and Dunlop lakes in spring and progressively increasing transparency in Quirke Lake until early fall, when it was almost twice that in Dunlop Lake (Fig. 3). Transparency in Pecors Lake, although measured only three times in 1966, apparently was intermediate between Quirke Lake and Dunlop Lake.

The times of formation and disruption, and the positions, of the thermoclines in the three lakes generally were similar (Fig. 4).

The greatest difference between the contaminated and unaffected lakes, considering both optical and thermal characteristics, was in the position of the euphotic zone with respect to the thermocline (the depth of 1% incident light approximated the lower limit of the euphotic zone). The euphotic zone extended well into the hypolimnion in Quirke and Pecors lakes but was confined to the epilimnion and thermocline in Dunlop Lake (Fig. 4).

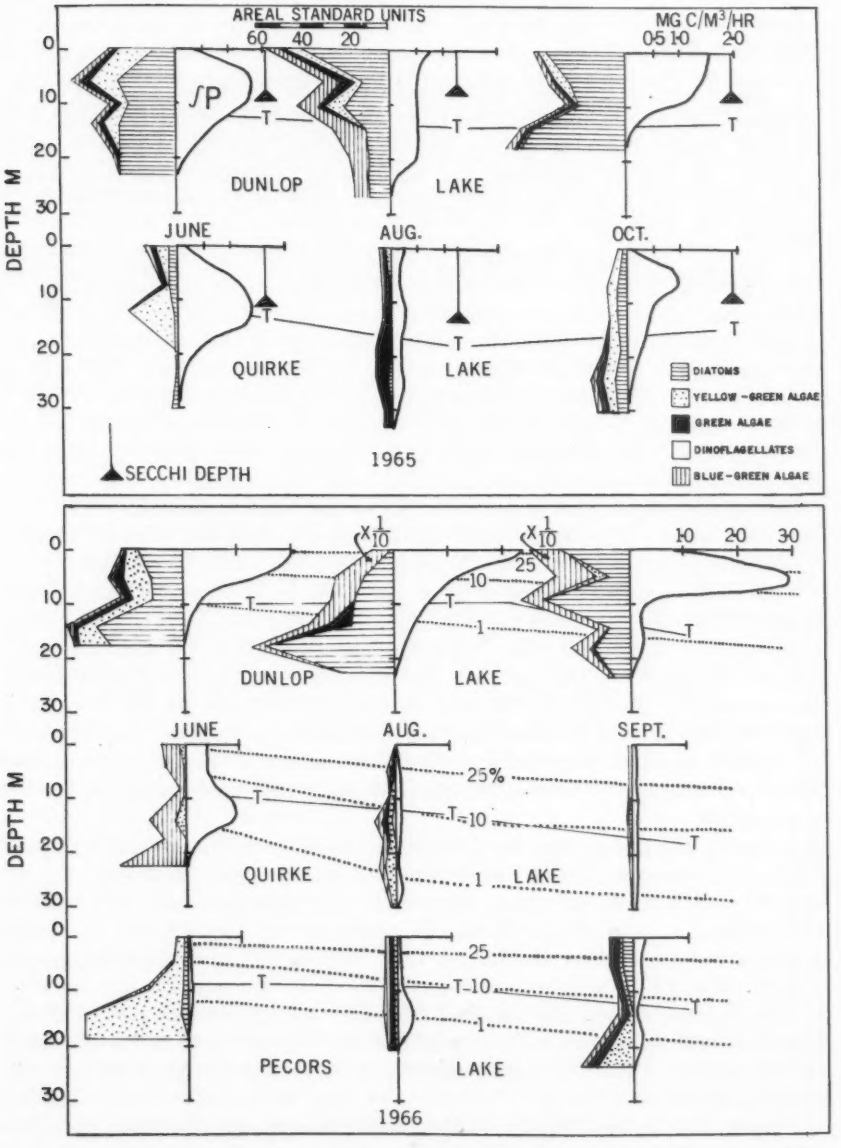

FIG. 4. Vertical variations in carbon assimilation (mg C m^{-3} hr^{-1}) and standing stocks of algae (asu) by taxonomic groups determined on visits to Dunlop and Quirke lakes in 1965 and to each of the lakes in 1966. Secchi-disc depths in 1965, isophots in 1966 (dotted lines), and the general positions of the thermocline (T) in both years are shown.

PHYTOPLANKTON COMMUNITIES

Standing Stocks

Differences in amounts and compositions of standing stocks of phytoplankton were observed in the three lakes. Phytoplankton populations decreased in order from Dunlop to Quirke to Pecors lakes. In 1966, average numbers were 62, 30, and 21 organisms per milliliter in Dunlop, Quirke, and Pecors lakes, respectively. Corresponding values in 1967 were 206, 168, and 31 organisms per milliliter.

A single prominent similarity in species composition was noted between the unaffected and one of the affected lakes. In Dunlop and Quirke lakes in 1966, an initial period of phytoplankton development during April was characterized by the predominance of the blue-green alga *Aphanizomenon flos-aquae* var. *gracile*, the higher numbers occurring in Dunlop Lake. In this lake, the alga was most abundant at intermediate depths (10–12 m), whereas in Quirke Lake it was most numerous at lower depths (18–22 m). In both lakes the alga gradually declined until the middle of May, when it disappeared. As the sampling program was not initiated until the middle of May in 1967, there was no record of an early spring blue-green pulse in the lakes that year. However, some *A. flos-aquae* var. *gracile* were found in the May 16 samples from Dunlop Lake but not in those from Quirke and Pecors lakes.

Several notable differences were observed in the species composition (Table 3) of phytoplankton between the unaffected and contaminated lakes. Firstly, many species of the Chrysophyceae, Myxophyceae, and Bacillariophyceae developed in Dunlop Lake but were absent or occurred in extremely low numbers in Quirke and Pecors lakes. Secondly, several species were found regularly only in the contaminated lakes. Further examination of the differences in standing stocks of phytoplankton between the unaffected and contaminated lakes more adequately reflects the impact of acid mine wastes on phytoplankton communities.

During the period May to July 1966 and May to August 1967, Dunlop Lake was characterized by moderate to high numbers of Chrysophyceae including *Dinobryon divergens*, *D. sociale*, *D. bavaricum*, *D. Vanhoeffenii*, *D. cylindricum*, and *D. sertularia*. In addition, *Chrysosphaerella longispina* was observed during May and June of 1967. Highest numbers of Chrysophyceae were found at 10–14 m in both years. With minor exceptions (e.g., low numbers of *D. sertularia*, *D. cylindricum*, and *D. sociale* in June and August in Quirke Lake and of these species and *D. bavaricum* in May and June in Pecors Lake), representatives of the Chrysophyceae were rarely encountered in the contaminated lakes.

From the end of August to the termination of the sampling period in 1966 and 1967, standing stocks of phytoplankton in Dunlop Lake consisted of moderate to high numbers of four species of *Chroococcus*, three species each of *Aphanothece*, *Dactylococcopsis*, *Coelosphaerium*, and *Merismopedia*, two species each of *Aphanocapsa* and *Rhabdoderma*, and *Gomphosphaeria lacustris*.

Greatest numbers of these Myxophyceae occurred above the thermocline but no near-surface concentrations were observed. In contrast, in Quirke and Pecors lakes during the late spring, summer, and fall of 1966 and 1967, standing stocks of phytoplankton were composed almost entirely of the blue-green alga *Plectonema notatum* of the order Hormogonales. This species appeared evenly distributed vertically to the maximum depth sampled in Quirke Lake but was most abundant at 10–18 m in Pecors Lake. Although some species of the Chroococcales were found in Quirke and Pecors lakes, numbers of most were extremely low. Of particular significance were the higher numbers of *Chroococcus Prescottii*, *Dactylococcopsis Smithii*, and *Rhabdoderma lineare* in the affected lakes than in Dunlop Lake. Apparently, these species are adapted to acid waters (Prescott, 1951).

The major diatoms in both years in Dunlop Lake were *Cyclotella bodanica*, *C. glomerata*, *Asterionella formosa*, *Tabellaria fenestrata*, and *Melosira italica*. Moderate numbers of the Bacillariophyceae were encountered over the entire sampling period, unlike representatives of the Myxophyceae and Chrysophyceae, which varied seasonally. In both years, maximum numbers of diatoms appeared in samples collected from 18 to 22 m. In contrast, in Quirke and Pecors lakes diatoms (including *Synedra ulna*, *S. pulchella*, and *T. fenestrata*) were either evenly distributed in low numbers throughout the euphotic zone or were absent. Of particular interest in the contaminated lakes was the occurrence of *Eunotia pectinalis* var. *minor*. Although this species never attained dominance, it was encountered in all samples taken from the contaminated lakes; however, it was never found in the unaffected lake. Patrick and Reimer (1966) indicate that this diatom tolerates higher calcium concentrations than other members of the genus and may be found in acid waters.

Species of Chlorophyceae occurred regularly in all three lakes; however, numbers generally were higher in the affected lakes. In fact, on August 31, 1967, in Pecors Lake, *Chlamydomonas* spp. were dominant, together with the blue-green alga *P. notatum*.

A tabulation of the numbers of cells of each species in each sample and areal measurements of cell biomass in "productivity" samples is available from the authors on request.

Species Diversity

With one exception (May 16, 1967, in Quirke Lake) mean indices of diversity in the contaminated lakes were lower than those in the unaffected lake (Table 4). A trend toward increased diversity during the late summer and fall months in the three lakes was observed. The index of diversity within the three communities was inversely related to standing stocks of phytoplankton. For example, in March and April 1966, the phytoplankton communities in Quirke and Dunlop lakes were characterized by high standing stocks of *A. flos-aquae* var. *gracile*, and, during this period, indices of diversity were relatively low at 2.74 and 3.02 in Dunlop Lake and 1.66 and 1.53 in

TABLE 3. Species of phytoplankton found in Dunlop, Quirke, and Pecors lakes during the spring, summer, and fall months, 1966 and 1967.

	Dunlop L.	Quirke L.	Pecors L.
Bacillariophyceae			
Asterionella formosa Hassal	×	×	×
A. gracillima (Hantzsch) Heiberg		×	
Cocconeis sp.			×
Cyclotella bodanica Eulenstein	×	×	
C. glomerata Bachmann	×		×
C. ocellata Pantocsek	×		
Cymbella affinis Kützing			×
Diatoma vulgare Bory			×
Eunotia pectinalis var. *minor* (Kütz.) (Rabenhorst)		×	×
Fragilaria crotonensis Kitton	×	×	×
Gomphonema sp.		×	
Melosira italica (Ehr.) Kützing	×	×	×
Navicula spp.	×	×	×
Nitzschia spp.	×	×	×
N. gracilis Hantzsch	×		
Pinnularia sp.		×	
Pleurosigma sp.			×
Surirella sp.	×		
S. angustata Kützing	×		
Synedra pulchella (Ralfs) Kützing	×	×	×
S. ulna (Nitzsch) Ehrenberg		×	
Tabellaria fenestrata (Lyngb.) Kützing	×	×	×
T. flocculosa (Roth) Kützing		×	×
Chlorophyceae			
Actinastrum sp.		×	
Ankistrodesmus sp.	×	×	
A. falcatus (Corda) Ralfs			×
A. fractus (West and West) Brunnthaler			×
Arthrodesmus sp.	×	×	
A. triangularis var. *subtriangularis* (Borge) W. and G. S. West	×		
Carteria sp.		×	
Chlamydomonas spp.	×	×	×
Chlorella ellipsoidea Gerneck	×	×	×
C. vulgaris Beyerinck		×	
Closterium sp.		×	×
Coelastrum sp.		×	×

	Dunlop L.	Quirke L.	Pecors L.
Chrysophyceae			
Chrysosphaerella longispina Lauterborn	×		
Dinobryon bavaricum Imhof	×		×
D. cylindricum Imhof	×	×	×
D. sertularia Ehrenberg	×	×	×
D. sociale Ehrenberg	×	×	×
D. sociale var. *americanum* (Brunn.) Bachmann	×	×	
D. Vanhoeffenii (Krieg.) Bachmann	×		
Mallomonas acaroides Perty	×		
Rhizochrysis limnetica G. M. Smith	×		
Synura uvella Ehrenberg	×		
Cryptophyceae			
Cryptomonas erosa Ehrenberg	×		×
C. ovata Ehrenberg	×		×
Dinophyceae			
Ceratium hirundinella (O. F. Muell.) Dujardin	×		
Glenodinium quadridens (Stein) Schiller		×	
Peridinium cinctum (Muell.) Ehrenberg	×		×
P. inconspicuum Lemmermann		×	
P. pusillum (Penard) Lemmermann		×	×
Euglenophyceae			
Euglena sp.	×	×	×
Trachelomonas sp.		×	
T. robusta Swirenko		×	×
Myxophyceae			
Anabaena sp.			×
A. spiroides var. *crassa* Lemmermann	×		
Aphanizomenon flos-aquae var. *gracile* (Lemmermann) Elenkin	×	×	×
Aphanocapsa elachista West and West	×		
A. elachista var. *conferta* West			

Chlorella ellipsoidea Gerneck	×	×	×
C. vulgaris Beyerinck		×	
Closterium sp.		×	×
Coelastrum sp.		×	×
C. microporum Naegeli	×		
Cosmarium sp.	×	×	
Crucigenia quadrata Morren	×	×	
C. rectangularis (A. Braun) Gay	×	×	×
Dictyosphaerium sp.	×		
D. pulchellum Wood		×	
Euastrum sp.	×		
Gloeocystis gigas (Kützing) Lagerheim	×		
G. vesiculosa Naegeli	×		
Golenkinia radiata (Chod.) Wille	×	×	
Kirchneriella sp.		×	
K. subsolitaria G. S. West	×		
Micractinium pusillum Fresenius	×		
M. quadrisetum (Lemmermann) G. M. Smith	×		
Mougeotia sp.	×	×	×
Nephrocytium limneticum (G. M. Smith) G. M. Smith	×		
Oedogonium sp.			×
Oocystis Borgei Snow	×		
O. parva West and G. S. West		×	
O. pusilla Hansgirg	×	×	×
Pediastrum Boryanum (Turpin) Meneghini	×		
Planktosphaeria gelatinosa G. M. Smith			×
Quadrigula closterioides (Bohlin) Printz	×		
Scenedesmus Bernardii G. M. Smith			×
S. bijuga var. *alternans* (Reinsch) Hansgirg		×	
S. quadricauda (Turpin) Brébisson	×		
Schroederia sp.			×
Selenastrum minutum (Naeg.) Collins		×	×
Sphaerocystis Schroeteri Chodat	×		×
Spirogyra sp.			×
Staurastrum sp.		×	×
S. Johnsonii West and G. S. West	×		
Tetraedron sp.		×	
T. minimum (A. Braun) Hansgirg			×
Ulothrix sp.	×	×	

Aphanocapsa elachista West and West	×		
A. elachista var. *conferta* West and West	×	×	
A. pulchra (Kützing) Rabenhorst	×		
Aphanothece clathrata G. S. West	×		
A. gelatinosa (Henn.) Lemmermann	×		
A. saxicola Naegeli	×		
Chroococcus dispersus (Keissler) Lemmermann	×	×	
C. limneticus Lemmermann	×		×
C. limneticus var. *carneus* (Chodat) Lemmermann	×		
C. Prescottii Drouet and Daily	×	×	×
C. turgidus (Kützing) Naegeli	×		
Coelosphaerium Kuetzingianum Naegeli	×		
C. Naegelianum Unger	×		
C. pallidum Lemmermann	×		
Dactylococcopsis acicularis Lemmermann	×	×	
D. fasicularis Lemmermann		×	×
D. rhaphidioides Hansgirg	×	×	×
D. Smithii Chodat and Chodat	×	×	×
Gloeothece rupestris (Lyngb.) Bornet	×	×	×
Gloeotrichia natans (Hedwig) Rabenhorst		×	
Gomphosphaeria spp.	×		
G. lacustris Chodat			×
Merismopedia convoluta Brébisson	×		
M. glauca (Ehrenberg) Naegeli	×		
M. tenuissima Lemmermann	×		
Oscillatoria subbrevis Schmidle		×	
Plectonema notatum Schmidle		×	×
Rhabdoderma Gorskii Woloszyńska	×		
R. lineare Schmidle and Lauterborn	×	×	×
Xanthophyceae			
Botryococcus Braunii Kützing	×	×	×
B. protuberans W. and G. S. West	×		

TABLE 4. Mean and extreme values of the index of diversity (I) for each sampling date in each of the lakes, 1966–67. Each arithmetic mean is based on five to six samples collected from a series of depths. See text for method of calculation.

Date	Dunlop L.			Quirke L.			Pecors L.		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
				1966					
Mar. 31	2.74	1.60	4.10	1.66	0.81	2.53			
Apr. 29	3.02	2.43	3.63	1.53	0.99	2.03			
May 19	3.59	2.60	4.36	4.85	0.73	5.87			
June 14	4.50	3.09	6.66	1.10	0.56	1.54			
June 28	5.99	4.67	7.69	1.63	0.84	3.41			
June 29							2.87	1.91	3.71
July 13	6.91	5.06	8.93	1.81	1.17	2.83			
July 28	4.94	3.50	5.88	3.21	1.12	6.49			
Aug. 13	6.68	4.79	7.79	2.30	0.99	4.05			
Aug. 17							2.89	1.69	3.94
Aug. 30				2.43	1.60	3.14			
Sept. 26	6.74	5.33	7.79	2.11	0.52	5.07			
Sept. 28							3.66	2.69	4.93
Oct. 20	7.53	5.93	9.27	5.12	3.17	6.31			
Nov. 15	9.59	7.40	11.59	6.91	4.68	8.04			
				1967					
May 16, 17	3.61	2.22	4.79	3.81	3.33	4.31	3.60	3.46	4.01
June 6, 7	4.57	2.43	8.04	2.89	2.00	3.93	2.80	2.32	4.00
June 27, 28	5.88	4.65	7.30	3.30	2.65	3.98	3.80	2.15	4.90
July 13, 14, 15	6.57	4.42	8.88	3.37	2.41	4.48	3.10	2.53	4.46
July 27, 28	9.02	6.50	12.25	2.34	0.68	4.83	4.00	0.91	7.63
Aug. 28, 31	7.09	5.07	9.41	4.03	1.77	5.47	3.80	3.21	5.00
Sept. 13	7.81	5.14	9.71	4.25	3.26	5.64			
Oct. 2, 3	8.22	7.52	9.20	4.09	2.79	5.48	3.30	2.07	4.69

Quirke Lake. An exception occurred in Dunlop Lake in the summer when both the standing stocks and indices of diversity were high.

PRIMARY PRODUCTIVITY

PRODUCTIVITY PER UNIT VOLUME

Primary productivity per unit volume generally was greater in the unaffected lake than in the contaminated lakes (Fig. 4). Hourly rates of carbon assimilation were as great as 2–3 mg C m^{-3} in Dunlop Lake during the development of blue-green algae in 1966. Rates exceeding 1 mg C m^{-3} hr^{-1} were common in Dunlop Lake and rare in Quirke Lake, whereas the maximum rate observed in Pecors Lake was 0.4 mg C m^{-3} hr^{-1}.

Although integrals (mg C m^{-2} hr^{-1}) should be used when comparing productivities of the lakes, an examination of volume-based rates with corresponding concentrations of nutrients, light intensity, and standing stocks

of algae is informative. Inorganic carbon appeared to be the most important of the nutrients in controlling productivity. This variable, light intensity, and algal standing stock were related in multiple regression to primary productivity. The multiple correlation coefficient was 0.70, and the simple correlation coefficients were 0.52 for algal stock, 0.36 for inorganic carbon, and 0.32 for light intensity. The estimating equation is

$$PP = -0.9065 + 0.0045A + 0.2701C + 1.4007 \log_e I$$

where PP is primary productivity in mg C m^{-3} hr^{-1}, A is algal standing stock in asu per milliliter, C is inorganic carbon in mg C $liter^{-1}$, and I is light intensity in klux.

Areal Productivity

The two contaminated lakes had lower areal primary productivities than the unaffected lake. Means of primary productivity measurements in Dunlop, Quirke, and Pecors lakes were 12.6, 7.0, and 3.3 mg C m^{-2} hr^{-1}, respectively. Extrapolation to daily rates gave means of approximately 130, 70, and 30 mg C m^{-2}.

All of the lakes should be classified as oligotrophic on the basis of absolute levels of primary productivity (see Wetzel, 1964, table 10, and Strickland, 1960, table 9, for a comparison of many fresh and marine waters). However differences occurred in the vertical distribution of productivity between the unaffected and contaminated lakes. Findenegg (1964) considered that "... the shape of the vertical assimilation curve is very much more informative with regard to the degree of eutrophication than is the amount of organic matter produced per surface unit." Findenegg's Type I curve, with near-surface maximum and decrease in productivity with depth corresponding to the exponential decrease in light, was characteristic of Dunlop Lake except in August 1965 (Fig. 4). In contrast, the contaminated lakes generally showed Findenegg's Type II curve, an orthograde distribution of assimilation with light inhibition in the uppermost layer and no pronounced optimal depth of photosynthesis through a deep euphotic zone, which evidently indicates that scarcity of nutrients rather than decreasing light limits photosynthesis. Quirke Lake showed this type on several occasions, although vertical assimilation was obviously of Type I in June 1965 and approached that of Type I in October 1965 and June 1966. The Type II curve was typical of Pecors Lake, but above-average assimilation occurred in the hypolimnion at very low light intensity (1% of incident light) in general in Pecors Lake and in Quirke Lake each midsummer. The greater concentration of inorganic carbon and perhaps other nutrients and growth factors in the hypolimnion appeared to be responsible.

The 15 areal estimates of productivity in all lakes were related to the corresponding amounts of inorganic carbon and algae in the euphotic zone (Fig. 5). In multiple regression analysis 63% of the variability in primary

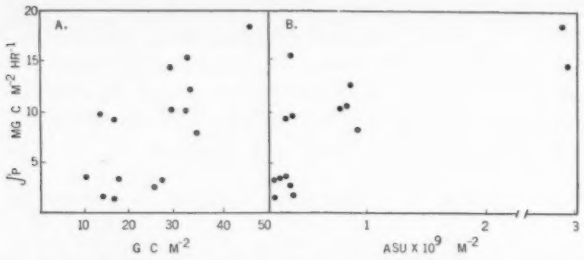

FIG. 5. Relationships between areal primary productivity and (A) inorganic carbon in the euphotic zone and (B) algal standing stock in the euphotic zone. Results of regression analysis are given in the text. The data on productivity and standing stock appear in Fig. 4. Inorganic carbon in the euphotic zone is shown in Fig. 2.

productivity was accounted for by variations in standing stock of algae and concentrations of inorganic carbon. The estimating equation is

$$PP = 10.5495 + 2.3813 \log_e A + 0.1996 C$$

where PP is in mg C m^{-2} hr^{-1}, A is asu $\times$ 10^9 m^{-2} of phytoplankton, and C is inorganic carbon in mg C m^{-2}.

BIOASSAYS

The addition of 6 mg per liter of inorganic carbon to Quirke Lake water doubled the rate of primary productivity in the experimental bags (Table 5). The addition of phosphate did not produce an increase. Addition of phosphate and inorganic carbon, alone and in combination, increased productivity of Dunlop Lake water, the combination giving the greatest increase. Although this bioassay experiment was carried out only once, it pointed to the importance of inorganic carbon in epilimnetic waters, at least in midsummer, in both Quirke and Dunlop lakes. The lack of response to phosphate in Quirke Lake

TABLE 5. Carbon assimilation (mg C m^{-3} hr^{-1}) in duplicate subsamples of Quirke and Dunlop lake waters removed from plastic bags to which nutrient additions had been made as indicated, August 13, 1966.

Addition	Quirke L.	Dunlop L.
None	0.13	0.38
	0.08	0.93
Phosphate (*0.2 mg PO_4 liter^{-1}*)	0.19	1.11
	0.13	1.21
Inorganic carbon (*6 mg C liter^{-1}*)	0.24	1.88
	0.23	1.45
Inorganic carbon and phosphate (*0.2 mg PO_4 liter^{-1} and 6 mg C liter^{-1}*)	0.27	3.73
	0.25	4.00

may have occurred because of the greater limiting role of inorganic carbon in this contaminated lake or preference of the phytoplankton species present at the time for low concentrations of phosphate.

The laboratory experiment showed increasing assimilation with increasing inorganic carbon concentrations up to 5 mg per liter whether phosphate had been added or not (Fig. 6). Assimilation appeared to reach an asymptotic level of 0.7–1.0 mg C m^{-3} hr^{-1} at about 5 mg C $liter^{-1}$ in water from both lakes. The interaction of phosphate and carbon did not give the greatest assimilation in Dunlop water in the laboratory as it had done in the polyethylene bags.

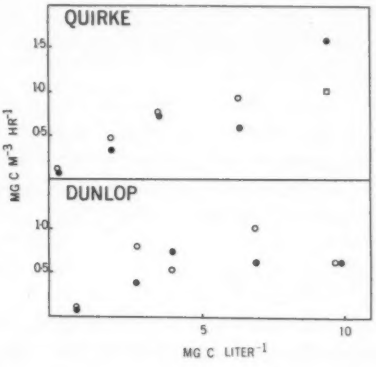

FIG. 6. Relationship between carbon assimilation by phytoplankton and concentration of inorganic carbon after additions of $NaHCO_3$ in in vitro bioassays of Quirke and Dunlop lake waters. Phosphorus (0.2 mg PO_4 $liter^{-1}$) was added to one-half of the samples (solid circles).

DISCUSSION

CHANGES IN LAKE CHEMISTRY

Differences in chemical composition between the lakes affected by mine wastes and the unaffected one can be related to various processes in the extraction of uranium and subsequent treatment of wastes. For example, sulphuric acid is used in leaching milled ore, nitric acid in eluting uranium from ion-exchange resins, and lime in neutralizing "barren" solution. These processes account for the increased concentrations of SO_4^{-2}, NO_3^{-1}, and Ca^{+2}.

Acid waste water is now neutralized prior to impoundment in tailings areas, although this was not done in the earliest operations. However, effluents from the ponds to the main lakes have a low *p*H, probably due to bacterial oxidation of metallic sulphides in the tailings, and provide the high free mineral acidity (Table 1) characteristic of the contaminated lakes.

Low concentrations of inorganic carbon were associated with low *p*H (Fig. 2). The low inorganic carbon evidently resulted from the reduced solubility of CO_2 and its loss from the epilimnion stirred by wave action and possibly by reduced regeneration of CO_2 from the sediments.

Other nutrients were in greater supply in the contaminated lakes than in the unaffected lake (e.g., nitrate) or occurred in concentrations similar to those in the unaffected lake (e.g., phosphorus and silica). Heavy metals did not attain high concentrations in the contaminated lakes.

Abundance and Distribution of Phytoplankton

The reduced standing stocks of phytoplankton appeared to be related to the effects of free mineral acidity in the contaminated lakes, including the shortage of inorganic carbon. Other nutrients examined apparently occurred in concentrations comparable with those in the unaffected lake.

In Dunlop Lake greater numbers of phytoplankton were found higher in the euphotic zone than in Quirke or Pecors lakes where the algae either were evenly distributed throughout the euphotic zone or were most abundant in the lower thermocline and in the hypolimnion. The latter distribution provided some evidence of more favourable conditions in the lower euphotic zone in contaminated lakes, where greater concentrations of inorganic carbon were available.

Similarities in species composition occurred in the three lakes, but the communities of these lakes clearly had individual characteristics. Modifications of communities in adapting to conditions of high salts and low *p*H were apparent in the resemblance of the communities of Quirke and Pecors lakes, which otherwise are least alike (e.g., in size and depth).

Species Diversity

The reduction in the population of phytoplankton in the contaminated lakes appears to be due to the elimination of some species and the drastic reduction in the numbers of other species as well as to reduction in numbers of surviving species. Also, when higher numbers were attained in the contaminated lakes, diversity did not increase. For example, the numbers of phytoplankton were relatively high in Quirke Lake in July in both 1966 and 1967, but species diversity was low because *P. notatum* predominated. In contrast, several species comprised the large population that developed in Dunlop Lake each summer. A greater degree of community organization is indicated in the unaffected lake, whereas modification of the environment in the contaminated lakes appears to lead to lower organization and to the general occurrence of opportunistic species like *P. notatum*. The development of an opportunistic species, *A. flos-aquae* var. *gracile*, in the unaffected lake, concurrent with low species diversity, was confined to the initial spring pulse.

The cyclical seasonal trend in species diversity, low in the spring with a general increase toward fall (Table 4), may be of general occurrence in these northern Ontario lakes. Although this cycle is of interest from the viewpoint of ecosystem succession, maturity, and organization, we have not pursued the matter in the present study.

Primary Productivity

The importance of inorganic carbon in limiting primary productivity was apparent in the in situ areal and volumetric measurements, in the laboratory bioassays, and in the bioassays carried out in polyethylene bags in the lakes.

Although all evidence from the study points to the limiting role of inorganic carbon in contaminated lakes, the importance of other factors cannot be discounted (e.g., changes in the ratio of monovalent and divalent ions and possible changes in concentrations of dissolved organic substances including growth factors).

Generally, Dunlop Lake was more productive than the contaminated lakes, but Quirke Lake had rates similar to those in Dunlop Lake on three occasions (Fig. 4) when the flow from Dunlop Lake to Quirke Lake was high. Possibly the inflow of water with normal content of inorganic carbon maintained primary productivity in Quirke Lake comparable with that in Dunlop Lake. Pecors Lake, which is supplied by water displaced from contaminated lakes and is generally low in inorganic carbon, was characterized by low standing stocks of phytoplankton and low rates of productivity.

A compensatory mechanism acted to reduce but did not eliminate the loss of inorganic carbon from the contaminated lakes. The reduction in phytoplankton, among other possible factors, led to an increase in the depth of the euphotic zone, which extended into the hypolimnion in Quirke and Pecors lakes (Fig. 3, 4). The average standing stocks of phytoplankton in Dunlop Lake at times when productivity was measured was 68 asu. In Pecors and Quirke lakes standing stocks averaged 7 and 18 asu, that is, 10 and 24% of the phytoplankton in Dunlop Lake. The average depths of the euphotic zone in Pecors and Quirke lakes at these times were 110 and 130% of that in Dunlop Lake where it was 22 m. Therefore, any compensating effect of lower concentrations of phytoplankton in producing a deeper euphotic zone appeared to be of limited significance. However, an extension of the euphotic zone into the hypolimnion, where greater concentrations of inorganic carbon occurred (Fig. 2), might be expected to enhance this mechanism if sufficient light remained. The region of overlap of the hypolimnion and euphotic zone received 1–10% of incident light, and a large proportion of the total carbon was assimilated at a very low (±1%) light intensity in the contaminated lakes (Fig. 4). However, because the light was so reduced in the hypolimnion, the mechanism as described above fell far short of compensating for the reduction in both species diversity and abundance of the phytoplankton. Nonetheless, the most efficient use of inorganic carbon in the contaminated lakes probably was the use of carbon held in the hypolimnion, much of which might otherwise have been lost from the lakes at times of complete circulation.

We conclude that inorganic carbon limited primary productivity in lakes contaminated by acid mine wastes. Regeneration of inorganic carbon probably is impeded in these lakes and carbon is cycled inefficiently, much being lost at overturn and from the epilimnion during stratification. Outside sources of inorganic carbon appear to be of extra importance to contaminated lakes. The reduction in primary productivity in lakes contaminated by acid mine wastes would be expected to affect higher trophic levels adversely and to delay the removal of radionuclides to the deep sediments.

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Effects of Water Currents on Distribution of Walleye Larvae in Oneida Lake, New York

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HOUDE, E. D., AND J. L. FORNEY. 1970. Effects of water currents on distribution of walleye larvae in Oneida Lake, New York. J. Fish. Res. Bd. Canada 27: 445–456.

Distribution of walleye (*Stizostedion vitreum vitreum*) larvae in Oneida Lake was followed in 1960–67 from the time 200 million 1- to 3-day-old hatchery larvae were stocked until dispersal was complete 10–15 days later. The distribution of prolarvae indicated a high proportion of the population originated from stocking. Newly stocked larvae concentrated near bottom and were transported by bottom currents into the western third of the lake. Older larvae were most abundant near the surface after yolk absorption and they were transported east by surface currents generated by the predominantly westerly winds. Distribution of larvae 8–9 days after release in 1966 and 1967 was similar to the distribution predicted from water current and wind velocity data. Larvae were dispersed further east in 1967, when winds were strong, than in 1966, when wind velocity was low.

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INTRODUCTION

WALLEYE (*STIZOSTEDION VITREUM VITREUM*) larvae are limnetic during the larval stage (Faber, 1967), and consequently are subject to transport by water currents. Spatial distribution of these larvae may be important in determining survival, as suggested for many marine fishes (Hjort, 1914; Settle, 1943; Walford, 1938). Newly hatched walleye larvae average 7.0–7.5 mm total length (TL) and have been described by Norden (1961). Larvae are photopositive from the time of hatching through the postlarval stage, but a relatively large yolk sac and poor swimming ability of newly hatched larvae allow the photopositive response to be only partially manifested. Prolarvae in aquaria swim in a vertical climb towards the surface, rotate head down, and sink to the bottom, where they may rest from a few seconds to many minutes before repeating the swimming attempts. The yolk is completely absorbed by 9.5 mm TL, and larvae then swim continuously near the surface of the aquarium except in complete darkness, when they rest on the bottom.

About 200 million 1- to 3-day-old walleye larvae are stocked annually in Oneida Lake. These prolarvae are released in open water and there is a rapid dispersal from the stocking site. Later, most postlarval walleyes are found in bays along the south shore of the lake, where they remain until becoming benthic. Studies were conducted in 1966–67 to determine the role

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of water currents in transporting larvae from the stocking site and their influence on postlarval distribution.

Oneida Lake is a shallow eutrophic lake in central New York with a surface area of 207 km². It is 31.7 km long, averages 7 km wide, and has a mean depth of 6 m. The long axis of the lake is oriented east–west, exposing its full length to the predominantly westerly winds. Limnological features of the lake were described by Mt. Pleasant et al. (1961) and the fishes by Adams and Hankinson (1928). Juvenile and adult walleye populations were studied by Forney (1966, 1967), and Houde (1967) reported on the food of walleye larvae.

BACKGROUND

Preliminary studies in 1960–64 suggested a high proportion of the larval population originated from stocking. In years when prolarvae were released near Scriba Creek (Fig. 1), density was initially high in the western third of

FIG. 1. Catches of walleye larvae per 100 m² in Oneida Lake during and up to 14 days after release of larvae 1–3 days old at the Scriba Creek site, 1960–63. Each value is the average of two tows at depths of 2 and 5.5 m in each of two or more years.

the lake and there was a gradual eastward movement of postlarval walleyes along the south shore 4–7 days after stocking. In 1964, when larvae were stocked near Shackelton Point, prolarvae were abundant in the central part of the lake and highest densities of postlarvae occurred in bays east and west of the stocking site (Fig. 2). This localized distribution of larvae indicated few wild larvae were present and movement of stocked larvae from the release site could probably be followed by sampling intensively.

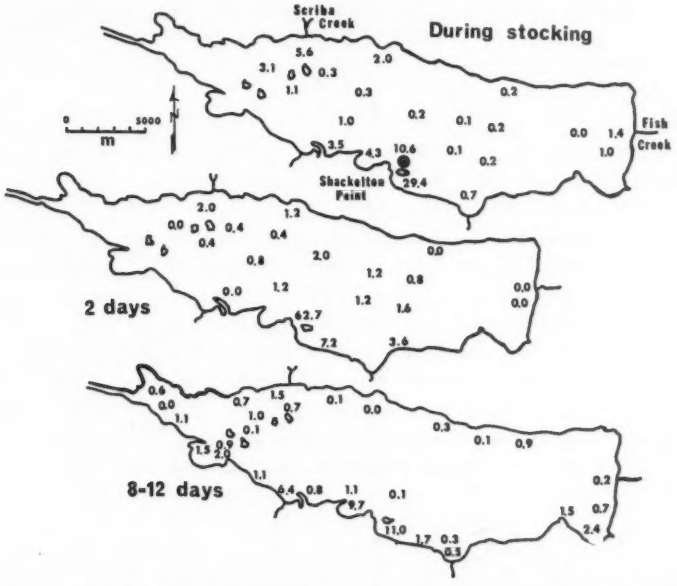

FIG. 2. Catches of walleye larvae per 100 m³ in Oneida Lake during and up to 12 days after release of 1- to 3-day-old larvae at the Shackelton Point stocking site in 1964. Each value is the mean of tows at 2- and 5.5-m depths.

The only large concentration of walleye prolarvae that could not be attributed to stocking was found near Scriba Creek in 1964, when larvae were released at Shackelton Point. These larvae presumably entered the lake from Scriba Creek, but it was uncertain whether they originated from eggs spawned in the stream or escapement from the hatchery. Few newly hatched larvae were caught over known spawning grounds in the lake or near Fish Creek, which is the only other tributary known to be a spawning stream.

The length distribution of larvae caught in meter nets provided additional evidence that stocked larvae predominated in the population. Development of eggs in the lake and Fish Creek was followed in 1959 and 1960, and it was estimated that eggs in these areas hatched 4–8 days after larvae were stocked (unpublished data). A total of 60 meter-net hauls before stocking yielded only 3 larvae, confirming that eggs spawned in the lake generally hatched

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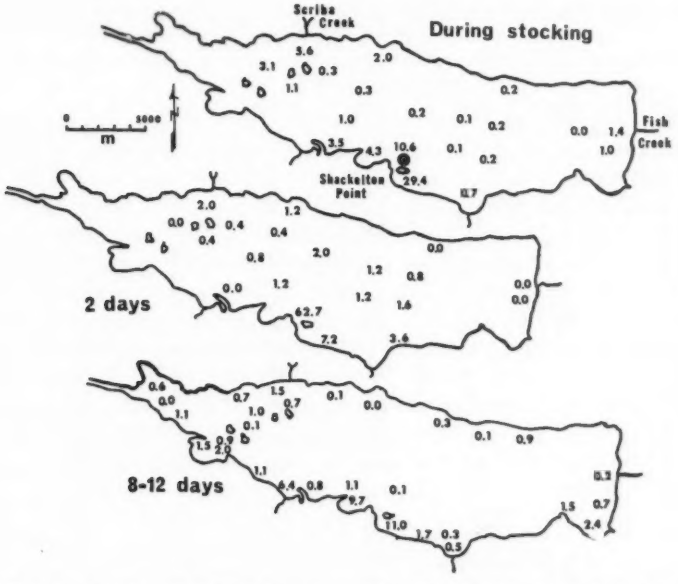

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after larvae were stocked. If wild larvae hatched later they would be younger and presumably smaller than those stocked. However, the length distribution of walleyes caught at 2-day intervals exhibited only a single mode, which shifted progressively from about 7 mm when larvae were stocked to 9–10 mm 10 days later. The nearly normal length distribution in 1966 and 1967 (Table 1) indicates that few young larvae were recruited to the population after stocking and most larvae collected during this study were stocked fish.

TABLE 1. Length distributions of larval walleyes caught in Oneida Lake 9–11 days after stocking in 1960–64 and 1966–67.

Length class (*mm*)	1960	1961	1962	1963	1964	1966	1967
6.6–6.7			3	1			
6.8–6.9			4	1			
7.0–7.1	1		3	8	3		
7.2–7.3	2		5	6	2		
7.4–7.5	1		7	12	1		
7.6–7.7	1		3	5	2		
7.8–7.9	1		9	12	1	1	1
8.0–8.1	5		13	12	3	0	0
8.2–8.3	3		9	20	3	2	1
8.4–8.5	4	1	13	15	2	4	2
8.6–8.7	2	6	12	26	4	10	8
8.8–8.9	8	12	30	57	11	18	19
9.0–9.1	6	12	29	97	18	24	17
9.2–9.3	7	20	40	82	20	23	17
9.4–9.5	13	33	35	35	31	21	13
9.6–9.7	5	22	21	13	24	7	1
9.8–9.9	2	8	16	6	21	1	
10.0–10.1	3	1	13		16		
10.2–10.3	2	0	1		5		
10.4–10.5	1	1			3		

MATERIALS AND METHODS

Dispersal of stocked larvae was followed in 1966 and 1967. About 100 million larvae were released at a buoy anchored 500 m off the mouth of Scriba Creek between 8:15 AM and 2:00 PM on May 19, 1966, and between 9:30 AM and 2:30 PM on May 16, 1967. Meter nets were towed at 2 m and near bottom at varying distances from the release point at intervals up to 24 hr after release to follow the dispersal of larvae. Calibrated flow meters were used to determine the volumes strained. Water currents were measured at six locations near the stocking site during the release of larvae. Both "semi-captive" and "free-drifting" drogues (Horrer, 1967; Putnam et al., 1949; Shulman and Bryson, 1961) were employed.

Drift of larvae from the 3rd day until the 8th or 9th day after stocking was predicted from wind records in 1966 and 1967. Water current velocities were estimated by two wind factors, the ratio of surface current velocity to wind velocity (Hutchinson, 1957) and the ratio of current velocity at 2- and 3-m depths to wind velocity. These wind factors were derived from drift card and drogue releases in 1965–67 (Houde, MS, 1968). Surface currents, for winds of 20–1600 cm/sec, were predicted by the hyperbola $Y = 1.868 + 273.166\,(1/X)$, where Y is the wind factor, expressed

as a percentage, regressed on X, the wind velocity in cm/sec. Similarly, current velocity to wind velocity ratios were plotted against wind velocity for currents at 2- and 3-m depths. For winds >150 cm/sec, which prevailed during the study, constants of 1.07 and 0.99% for 2 and 3 m, respectively, best described the relationship. Wind direction and velocity were recorded several times daily from readings on a three-cup anemometer mounted on a tower 18 m above lake level and approximately 0.4 km inland from the lakeshore. A Dwyer, hand-held wind gauge was used to measure wind velocity when water currents were being measured on the lake.

The predicted distribution of larvae 8–9 days after stocking was compared with the catch in Miller samplers (Miller, 1961). Four samplers at different depths were towed simultaneously at 42 randomly selected stations in 1966 and at 60 in 1967. Samplers were towed for 5 min at 12.6 km/hr and the mean volume strained was 8.4 m^3.

DRIFT OF PROLARVAE

Subsurface currents generated by the prevailing westerly winds were important in transporting larvae from the stocking site near Scriba Creek. Winds were light northwest on May 19, 1966, and west-northwest at high velocities of 6.7–17.0 m/sec on May 16, 1967, when larvae were stocked. Surface currents flowed east to southeast, and currents below 2 m moved counter to the wind in a westerly direction both years. Velocities near bottom (4–6 m) averaged 2.1 cm/sec in 1966 and 4.0 cm/sec in 1967. The catch of larvae in meter nets towed at 2 m and near bottom indicated an average density of 56.6 walleyes per 100 m^3 at 18 stations west of the stocking site and 16.1 per 100 m^3 at 7 stations east of the site 1–10 hr after larvae were released in 1966. Similarly in 1967, catches at stations 7–14, which were west of the stocking site (Fig. 3), averaged 14.6 larvae per 100 m^3, while stations 5, 6, and 15, which were to the east, averaged only 1.5 larvae per 100 m^3.

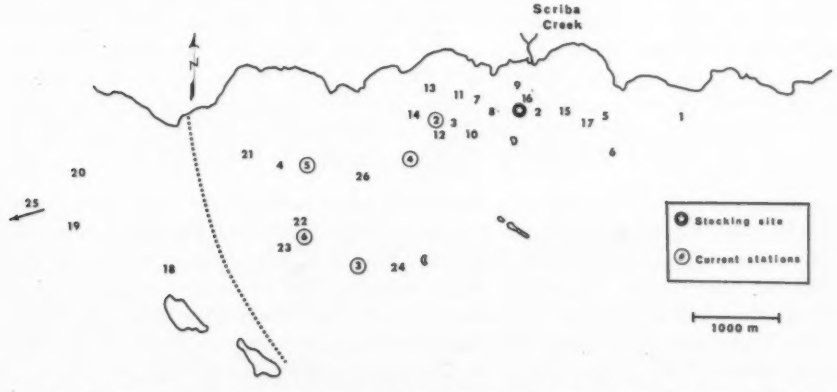

FIG. 3. Walleye larvae sampling stations and water current measuring stations on May 16 and 17, 1967. Current station 1 is at the stocking site. Stations 1–15 sampled on May 16, and 16–26 on May 17. Maximum drift of larvae after 24 hr predicted from bottom current measurements and indicated by broken line.

Larvae tended to be more abundant near bottom than at 2 m after stocking in 1966 and 1967 (Table 2). In a high proportion of the paired hauls largest

TABLE 2. Numbers of walleye larvae per 100 m^3 collected at two depths within 24 hr after stocking in a meter net haul at various stations[a] near the stocking site, 1966 and 1967.

	1966		1967	
Station	At 2 m	Near bottom	At 2 m	Near bottom
1	28	176	0	0
2	47	150	6	2
3	0	0	2	4
4	0	0	1	1
5	0	9	0	0
6	0	1	0	0
7	0	2	0	1
8	1651	1341	0	54
9	1	1	14	74
10	1	0	4	22
11	–	–	0	8
12	–	–	1	8
13	–	–	0	13
14	20	28	2	31
15	–	–	0	7
16	1	0	73	179
17	0	0	1	3
18	110	36	0	1
19	33	43	0	2
20	0	1	1	0
21	1	2	0	2
22	0	1	2	16
23	1	5	1	8
24	19	12	0	3
25	1	2	0	3
26	1	5	0	2
27	0	1	–	–

[a]Figure 3 shows the stocking site and sampling stations in 1967.

catches were in the bottom net, indicating most larvae were being transported by bottom currents flowing in a westerly direction. The highest single catch of larvae occurred at 2 m in 1966, but this catch was made at station 8, only 200 m from the stocking site, while stocking was in progress, and it seems likely that larvae had not yet sunk to the bottom. Excluding this observation, paired catches at the two depths differed significantly in 1966 ($P < .025$) and 1967 ($P < .005$) based on Wilcoxon's signed rank test.

Rate of drift of larvae was difficult to accurately estimate since some newly hatched walleyes were present in the stocking area before larvae were released. However, samples at four stations in 1967 indicated that prestocking density was less than 4 per 100 m^3 (Table 3) so higher catches would imply that larvae were being transported to a station. High densities of larvae were found in 1967 at stations 8 and 9 (Table 3) about 3 hr after stocking began.

TABLE 3. Walleye larvae per 100 m³ collected in meter nets at various stations on May 16–17, 1967. Stations are shown in Fig. 3. Stations 1–4 were sampled before stocking began at 9:30 AM on May 16. Catches are means from samples at 2 m and near bottom.

May 16			May 17		
Station	Time	Catch	Station	Time	Catch
1	7:30 AM	0.0	–	–	–
2	8:00 AM	3.9	17	8:00 AM	2.2
3	8:30 AM	3.2	18	8:30 AM	0.7
4	9:00 AM	1.0	19	8:45 AM	1.5
5	11:20 AM	0.5	20	9:00 AM	0.2
6	11:30 AM	0.0	21	9:10 AM	1.0
7	12:00 NOON	0.5	22	9:25 AM	9.1
8	12:10 PM	27.4	23	9:45 AM	4.4
9	12:20 PM	44.0	24	9:55 AM	1.7
10	1:45 PM	13.2	25	10:10 AM	1.5
11	2:00 PM	3.9	26	10:25 AM	1.0
12	2:20 PM	4.4	–	–	–
13	2:30 PM	6.4	–	–	–
14	2:45 PM	16.7	–	–	–
15	3:10 PM	3.9	–	–	–

Bottom currents slightly less than 4 cm/sec, the observed average velocity on May 16 (Table 4), would have carried larvae to these stations. Four to five hours after stocking, concentrations of larvae were found at stations 10 and 14. Currents of 4.8 and 6.4 cm/sec would have been required to carry larvae to these stations. Although these velocities are high, they are within the range observed on May 16. About 24 hr after stocking, a single concentration of larvae was located at stations 22 and 23. Larvae released at 9:30 AM on May 16 would have had to drift 3.6 cm/sec to reach these stations. The average of all current velocities measured on May 16 and 17 was 3.6 cm/sec — a velocity just adequate to transport larvae to stations 22 and 23.

TABLE 4. Average directions and velocities of bottom currents at stations 1–4 near the stocking site on May 16 and stations 5 and 6 on May 17, 1967. Results are mean values from several determinations at each station.

Station	Date	Depth (*m*)	Direction (*deg*)	Velocity (*cm/sec*)
1	May 16	4.5	287	3.10
2	"	4.0	215	2.50
3	"	6.0	274	7.75
4	"	6.0	274	2.50
5	May 17	5.5	265	2.50
6	"	6.5	300	3.60

DRIFT OF POSTLARVAE

The catches of walleyes at 2 and 5.5 m in 1960–63 suggested that there was a gradual eastward movement of the population along the southern shore beginning about 4 days after stocking (Fig. 1). This eastward movement may be explained by a change in the vertical distribution of larvae as they became older. Prolarvae were concentrated near bottom after stocking but postlarvae were most abundant in the upper 3 m of the water column as was illustrated by the depth distribution of larvae caught in 408 tows with Miller samplers 8–9 days after stocking in 1966–67:

Depth (*m*)	1	2	3	4	5	6
Mean catch per haul	0.49	0.65	0.43	0.26	0.03	0.00

Postlarvae would tend to drift eastward since surface currents usually flow east under the influence of the prevailing west winds.

Postlarvae apparently remained concentrated near the surface throughout the day. Vertical distributions during day and night were similar as shown by the total catches of larvae at two locations near Shackelton Point on three occasions in 1962 and 1963:

Depth (*m*)	No. larvae		
	Day	Night	Total
0–2	196	122	318
3–3.6	102	77	179
3.6–5.5	112	83	195
Total	410	282	692

The increased abundance of older larvae near the lake surface probably is related to absorption of the yolk sac and increased swimming ability. Tests of swimming ability (Houde, 1969) and observations on swimming of larvae in aquaria suggest that most larvae would be capable of swimming continuously near the surface 3 days after stocking.

Near-surface currents from May 21–27, 1966, and May 18–25, 1967, were estimated from wind records and their relation to larval distribution was examined. West winds predominated in both years (Table 5), but velocities were much higher in 1967. Current velocities at 0, 2, and 3 m were predicted from the wind factor relation (Table 6) for east and west winds, and the mean velocity in this depth range was obtained from the area under the regression of predicted current velocity against depth. Total east and west drift distances were estimated from the product of the mean velocity in the top 3 m and the number of hours of wind. The differences between the total east and west drifts predicted a net easterly drift at a rate of 49.5 m/hr in 1966 and 77.6 m/hr in 1967. The direction of drift probably was east-southeast since most winds originated from 271–315°. Assuming that larvae were scattered over

TABLE 5. Wind directions and velocities on Oneida Lake from 8:00 AM May 21 until 4:00 PM May 27, 1966, and from 8:00 AM May 18 until 4:00 PM May 25, 1967.

Year	Wind direction (*deg*)	No. hr wind	Velocity-hr (*cm/sec*×*hr*)	Mean velocity (*velocity-hr/hr* = *cm/sec*)
1966	1–180	60	11,920	198.7
	181–360	92	24,955	271.2
Total		152	36,875	242.6
1967	1–180	68	28,255	415.5
	181–360	104	51,028	490.7
	No wind	4	–	–
Total		176	79,283	450.5

TABLE 6. Data used to calculate currents and predict drift of larvae in Oneida Lake from the 3rd until the 8th day after stocking in 1966 and from the 3rd until the 9th day after stocking in 1967.

Year	Avg wind velocity (*cm/sec*) and direction	Wind factor			Predicted current velocity (*cm/sec*)			Mean current velocity in top 3 m (*cm/sec*)	No. hr wind
		0 m	2 m	3 m	0 m	2 m	3 m		
1966	271.25 W	2.90	1.07	0.99	7.87	2.90	2.68	4.48	92
	198.67 E	3.12	1.07	0.99	6.20	2.12	1.97	3.39	60
1967	490.65 W	2.50	1.07	0.99	12.27	5.25	4.86	7.91	104
	415.51 E	2.60	1.07	0.99	10.80	4.44	4.11	6.52	68

the western third of the lake by the 3rd day after stocking, they should have drifted much further east in 1967 than in 1966 (Fig. 4) if surface currents were important in transporting larvae.

Relative abundances of larvae in sectors A–D of the lake (Fig. 4) were estimated from the catches in Miller samplers 8–9 days after stocking in 1966 and 1967. Four samplers at different depths were towed at 6–18 stations in each sector and the mean catch per station was used as an estimate of sector density (Table 7). An index of abundance was calculated for each sector by multiplying the sector mean by the volume of the sector between the surface and 6 m, the range of depths that were sampled. These indices suggest that at 8 days after stocking in 1966 over half the larvae were in sector A at the western end of the lake and that 86% were within the boundaries of the predicted maximum drift for that year. A wider distribution was observed in 1967, when more than half the larvae were in sectors B and C in the central portion of the lake and 79% were within the predicted boundaries.

The higher proportion of larvae in sectors B and C in 1967 than in 1966 suggests that a greater eastward drift occurred in 1967, which agrees with distribution predicted from wind data. That most larvae had drifted less than the maximum predicted distance may be partly explained by the fact

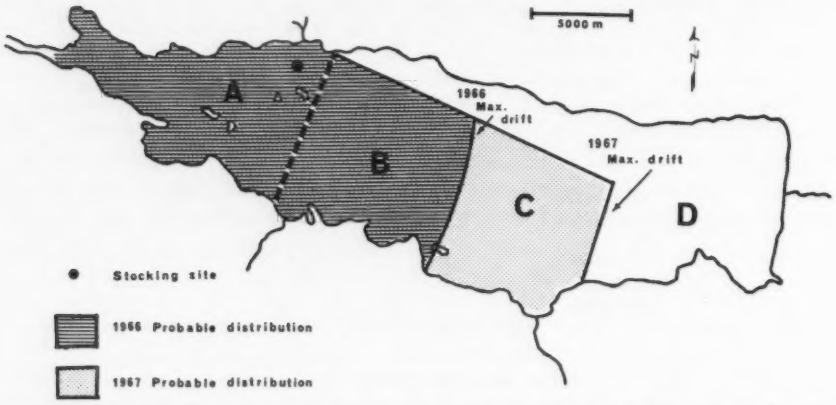

FIG. 4. Predicted maximum drift of walleye larvae in Oneida Lake during the 200 hr after stocking in 1966 and 224 hr in 1967. Broken line marks boundary for the western third of the lake from which maximum drift was predicted. Predictions were based on water current–wind velocity relation.

TABLE 7. Relative abundances of larval walleyes in four sectors of Oneida Lake 8 days after stocking in 1966 and 9 days after in 1967.

Year	Lake sector[a]	Relative density (*mean catch per station*)	Vol of sector (10^7 m^3)	Relative abundance	
				Area index	% of total
1966	A	4.8	18	86.4	51.7
	B	2.9	20	58.0	34.7
	C	0.8	20	16.0	9.6
	D	0.2	34	6.8	4.0
1967	A	1.5	18	27.0	23.9
	B	1.9	20	38.0	33.7
	C	1.2	20	24.0	21.3
	D	0.7	34	23.8	21.1

[a]Sectors are shown in Fig. 4.

that some larvae occurred below 3-m depth, where currents were often counter to the wind direction. Another factor that probably retarded eastward movement was the gradual concentration of larvae in bays along the south shore. Larvae in sector D at the eastern end of the lake may have originated from natural spawning in Fish Creek since it was unlikely that currents transported stocked larvae this distance in either 1966 or 1967.

CONCLUSIONS

Johnson (1969) observed that larval walleyes move offshore into open water after hatching and the young return to the littoral area after growing

to a length of 30–40 mm. Our studies show that newly hatched walleyes drift passively and currents over the spawning grounds would probably carry them into the limnetic zone. Although currents are important in the early dispersal of larvae from the spawning grounds, walleyes appear to be capable of regulating their distribution within 1 to 2 weeks after hatching.

Postyolk-sac larvae eventually would be concentrated in the eastern part of Oneida Lake if larvae continued to drift passively throughout the limnetic stage. But, this did not occur. At 8–10 days after stocking, greatest numbers of larvae were found in bays in the western part of the lake and along the south shore, where they remained concentrated until their transition to a demersal existence at 25–30 mm TL (J. L. Forney, unpublished data). Currents in two of the bays had eddying patterns during westerly winds, which might retain walleye larvae (Houde, MS, 1968) by mechanisms similar to those that concentrate pelagic larvae of marine fishes (Fraser, 1958; Saville, 1965). The initial transport of larvae into bays along the south shore may be facilitated by surface currents because winds are usually from the northwest. Active emigration inshore seems unlikely because larvae are about 9.5 mm TL at 8 days after stocking, and they have a sustained swimming ability of less than 3.0 cm/sec (Houde, 1969). Surface currents in open water are almost always of greater velocity. Possibly larvae can regulate their distribution at this stage by coordinated vertical migrations and active swimming, although the ability of walleye larvae to orient to currents while pelagic has not been demonstrated.

In large lakes the dispersal of walleye larvae from the spawning grounds will be determined by both hydrographic features of the lake and local wind-generated currents. Larvae may be transported into habitats more or less favorable for survival, depending on the pattern of winds during hatching. The fate of newly hatched larvae, carried by bottom currents into deep water, where low light intensity might impair feeding, would be of particular interest. Movement of young into the bays may affect survival, although our sampling has not demonstrated that the abundance of planktonic food or predators differed in bays and open water of Oneida Lake. If the bays in which pelagic larvae concentrate are also the nursery grounds of the young after they become demersal, then the initial dispersal may influence survival during the first year. Although many implications can be drawn from observations on larval distribution, its importance in determining year-class strength will remain speculative until mortality rates of larvae are accurately assessed.

ACKNOWLEDGMENTS

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A Review of the Morid Fish Genus *Lepidion* of the North Atlantic With First Records of *Lepidion eques* from the Western North Atlantic

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TEMPLEMAN, W. 1970. A review of the morid fish genus *Lepidion* of the North Atlantic with first records of *Lepidion eques* from the western North Atlantic. J. Fish. Res. Bd. Canada 27: 457–498.

Seventeen specimens of *Lepidion eques* (Günther) taken by the Fisheries Research Board of Canada St. John's, Nfld., Biological Station are apparently the first records of the species from the western Atlantic west of West Greenland. Comparison with specimens from the northeast Atlantic shows some differences in averages of meristic and morphometric characters, but with overlapping ranges in these characters.

Comparison of these North Atlantic *L. eques* with the Mediterranean *Lepidion lepidion* (Risso) revealed that although closely related, these species are separable on the bases of the smaller orbit diameter in relation to the head length and especially by the relatively larger postorbital length in *L. lepidion*. *Lepidion guentheri* (Giglioli) is distinctly different from these two species in some meristic and many morphometric characters. Lectotypes of *L. eques* and *L. guentheri* are designated. Various incorrect or doubtful records of *Lepidion* are discussed. Two giant lepidions reported recently from the northeast Atlantic as *L. guentheri* are tentatively assigned to *Lepidion schmidti* Svetovidov, a species hitherto known only from Japan.

The distributions of the North Atlantic species of *Lepidion* are discussed and information is provided on sexual maturity.

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INTRODUCTION

EIGHTEEN SPECIMENS of *Lepidion eques* (Günther, 1887) were taken, 17 in the northwest Atlantic and one west of Iceland (Fig. 1, 15; Table 1). The 17 individuals are apparently the first actual records of this species and genus from the northwest Atlantic.

Lepidion lepidion (Fig. 3) was described by Risso (1810) as *Gadus lepidion* (later, Risso, 1826, as *Lotta lepidion*) from specimens taken at considerable depths in the Mediterranean off Nice.

Lepidion guentheri (Fig. 4, 5) was described by Johnson (1862) and Günther (1862) as *Haloporphyrus lepidion*, synonymous with Risso's *G. lepidion*, from fish taken at Madeira, but was recognized by Giglioli (1880) and later by Günther (1887) and subsequent workers as a separate species. It was named by Giglioli as *Haloporphyrus guntheri*, the species name being written by Günther (1887) as *güentheri*.

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Swainson (1838) introduced the genus *Lepidion*, changing Risso's *G. lepidion* to *Lepidion rissoii* and later (Swainson, 1839) to *Lepidion rubescens* (= *L. lepidion* since the species name change was unwarranted). Günther (1862) replaced the generic name by *Haloporphyrus* but without argument, merely saying that the generic name of *Lepidion* was preoccupied.

Goode and Bean (1896) retained the generic name of *Lepidion*, stating that the name of *Lepidia* given to a genus of worms by Savigny in 1817 was sufficiently distinct from Swainson's *Lepidion*. Also Norman (1936) says that Swainson's *Lepidion* is not invalidated by *Lepidia* Savigny. Most but not all authors, however, up to the early 1950's used *Haloporphyrus*, but more recently the preference has been for *Lepidion*.

In 1966–67, Mr G. R. Forster of the Marine Biological Station at Plymouth caught on longline in very deep water at the mouth of the Bay of Biscay two very large specimens of *Lepidion*, which he attributed to *L. guentheri* (Forster, 1968). I have examined these at the British Museum and supplied meristic and morphometric data that show that on present evidence they are not *L. guentheri* but are closely related to or identical with *L. schmidti* Svetovidov, 1936 (Fig. 6).

MATERIALS AND METHODS

For the North Atlantic species of *Lepidion* the published descriptions are very inadequate. Few morphometric data are given, standard lengths are usually not given, and there is usually no indication of how the measurements were taken. Meristic data are most inadequate and often very inaccurate. Authors describing new species have usually given inadequate descriptions, and most others have not given meristic and morphometric data.

I have consequently carried out new meristic counts for 15 and body proportion measurements for 12 *L. eques* from the western Atlantic, meristic counts for 24 and body proportion measurements for 15 *L. eques* from the eastern Atlantic, and meristic counts and body proportion measurements for 13 *L. lepidion* and 8 *L. guentheri* (Table 3, Fig. 10–14; Templeman, MS, 1970, tables 2–6) so that future authors may have bases for comparison. Unless otherwise stated, only this author's counts and measurements are used in this paper. Additional tabular material and references useful for this *Lepidion* paper are to be found in Templeman (MS, 1970), which includes individual records of distribution and related data (as in Table 1) for all eastern Atlantic specimens of *Lepidion* known to the author and indicated in Fig. 15 of this paper; tabular details of meristic characteristics (summarized in Table 3) of individual lepidions; morphometric characteristics of individual lepidions used for Fig. 10–14, and some additional morphometric characteristics; also information on weights, food, and parasites of, and additional literature references to North Atlantic lepidions. Insofar as it is available, this paper will be sent to individual specialists on request to the author or to the St. John's Station.

In the following *Lepidion* specimens, meristic, and usually morphometric characteristics, were studied by the author. (For details see Templeman, MS, 1970, tables 2–6.)

Lepidion eques (Günther). British Museum (Natural History) (BMNH) 1887, 12.9.58–67: *Haloporphyrus eques* (types), Faroe Channel, *Knight Errant*, 1887 (11 fish, 129–271 mm standard length (SL), taken in 1880, 1882). BMNH 1905, 4.8.16–21: *H. eques*, W of Ireland SR 188, E. W. L. Holt, 1905 (5 fish, 264–336 mm SL). BMNH 1965, 6.22.21–27: *Lepidion guentheri*, *Ernest Holt* Cruise 3/1961 Sta. 42, W of Ireland, Rockall, R. W. Blacker (7 fish, 129–234 mm SL). St. John's Station, *A. T. Cameron*, 1962, W of Iceland (205 mm SL). St. John's Station, *A. T. Cameron* and *Investigator II* 1953–67, Newfoundland–Labrador area (15 fish, 139–305 mm SL).

TABLE 1. Records of captures of *Lepidion eques* by the Fisheries Research Board of Canada St. John's Station.

Item no.	Capture		Locality	Position		Depth (*m*)	Bottom temp (*C*)	No. specimens	Total length (*mm*)
				Lat N	Long W				
1	1953[a]	Oct. 5	E of Funk Is. Bank	50°05′00″	50°21′00″	549–552	3.54	2	242, 282
2	1958[a]	Oct. 4	Mouth of Hawke Chan.	53 14 00	52 08 00	550	4.00	1	283
3	1959	Sept. 8	SE of Cape Chidley	60 00 00	59 58 30	550–565	4.03	1	155
4	"	Sept. 30	E of Belle Is.	52 27 00	51 30 00	550–560	3.53	1	220
5	"	Oct. 2	E of Belle Is.	52 01 00	50 48 00	545–552	3.62	1	265
6	1960	Aug. 11	SE of Hamilton Inlet Bank	53 38 00	52 25 00	549–551	4.36	1	309
7	1962	July 30	W of Iceland	64 44 00	27 15 00	385–405	ca. 6[b]	1	226
8	1963	May 28	E slope Funk Is. Bank	51 24 00	50 08 30	543–558	3.36	2	197, 227
9	"	Sept. 9	E slope Funk Is. Bank	51 28 30	50 09 50	640–685	3.72	1	332
10	"	Sept. 13	Mouth of Hawke Chan.	53 26 30	52 20 15	490–550	3.91	1	188
11	1964	Apr. 7	E slope Funk Is. Bank	51 26 00	50 07 00	675–775	3.49	1	317
12	1964	Nov. 26	SE Grand Bank	45 23 45	48 24 45	640	3.99	1	227
13	1967	Nov. 1	E slope Funk Is. Bank	51 25 30	50 08 30	607–658	4.11	2	290, 309
14	1969	June 9	SE of Hamilton Inlet Bank	53 22 22	52 15 15	613–649	3.86	2	302, 333

[a]Specimens taken by the *Investigator II* by bottom trawl; others taken by the *A. T. Cameron* by bottom trawl.
[b]No temperatures taken but estimated from hydrographic sections for this area and season.

Lepidion lepidion (Risso). BMNH 86.8.4.6: *Lota lepidion* from Nice (230 mm SL). Museo Civico Genova, MSNG 7703, 31898 (2 fish, 230 and 235 mm SL). Muséum National, Paris, 4293, A2225, 86–275, 96–30, 98–913, 57–106, NW Mediterranean (6 fish, 116–256 mm SL). Musée Océanographique, Monaco, Sta. 392, Sta. 632, Sta. 1252 NW Mediterranean (4 fish, 163–263 mm SL).

Lepidion guentheri (Giglioli). BMNH 1862, 4.22.9: *Haloporphyrus lepidion*, Madeira, presented by J. Y. Johnson (lectotype of *L. guentheri*, 554 mm SL). BMNH 72.2.6.10: *H. lepidion* from W coast Spain and Portugal, 1870 (474 mm SL). Musée Océanographique Monaco, from the Azores 1895 (121 mm SL; also mentioned by Roule, 1919). Museu Municipal do Funchal, No. 3516, 3520, 3604, 21507, 22049, from Madeira (5 fish, 413–536 mm SL).

Lepidion sp. (*L. ?schmidti*). BMNH: *L. guentheri* (see Forster, 1968), from mouth of Bay of Biscay, 1966 and 1967 (2 fish, 1045 and 1127 mm SL).

The northwest Atlantic specimens were in good condition for the meristic characters, except for scales along the lateral line, and in fairly good shape for body proportions. All were preserved in 10% formalin and measured after a short time, up to about a day, in water.

All the eastern and north-central Atlantic specimens that I examined (except Maul's specimens from Madeira, which were in formalin, and Forster's *Lepidion* sp., which were in phenoxetol) were ex-alcohol and usually in fair to good condition.

Except where otherwise noted, morphometric data and meristic and other number counts (subsequently all called meristic counts) were obtained by the methods of Hubbs and Lagler (1958). Apart from total lengths and standard lengths, which are the shortest distances between parallel perpendicular planes, and any other distances especially noted, all measurements are direct distances between points.

Where suitable, the left side of the head and body was used for morphometric and meristic purposes. If the left side was unsuitable the right side was used.

All the *Lepidion* specimens that I examined from the North Atlantic had the typical *Lepidion* head with upper jaw extending in front of the lower, the first long dorsal ray and the first two rays of the pelvic fins prolonged into long filaments, small scales, a small patch of vomerine but no palatine teeth, a barbel, seven branchiostegal rays, a separate caudal fin, two dorsal fins, one anal fin with the anterior and posterior rays longest, and the posterior insertion of the anal anterior to that of the second dorsal.

In the first dorsal fin there is anteriorly to the first long ray a small spine or ray as in *Halargyreus* (Collett, 1905; Templeman, 1968) but far less likely than in *Halargyreus* to be noted externally, being usually concealed in the slight swelling anterior to the base of the first long ray of the first dorsal. It can be readily noted in a radiograph (Fig. 7, 8) and where I have not reported it for my specimens in Templeman (MS, 1970, table 2) the specimens were either too small for the radiograph to show fin rays clearly or the radiograph was unsuitable for distinguishing the rays of the first dorsal. In all radiographs of *Lepidion* specimens showing the first dorsal fin rays clearly, this small spine or ray was present and supported by a basal pterygiophore. Consequently where the first dorsal consists of one short and four longer rays (1+4) there are five associated pterygiophores and where the first dorsal is of the type 1+5 there are six associated pterygiophores.

Because the authors were not using radiographs, the presence of this small anterior spine or ray is rarely noted in the literature or included in the counts of the first dorsal fin rays. Exceptions are Vinciguerra (1883), who, for *L. lepidion*, says that the first ray of the first dorsal is rudimentary and hidden under the skin (this is also indicated in his excellent drawing); and, indirectly, Koefoed (1927), who, for a small specimen of *L. eques*, 72 mm long, states that the second ray in the first dorsal is the longest.

The first anal ray (Fig. 8) is usually relatively short, and in preserved specimens closely related to the second ray, and is likely to be missed if the skin covering this portion of the fin is not removed or if radiographs are not used. It is not shown in Holt and Calderwood's (1895; copied by Schnakenbeck, 1933) figure of *L. eques*. It is, however, shown in Maul's (1952) figure of *L. guentheri*. It is also easy to miss the most posterior anal ray, which often lies posteriorly

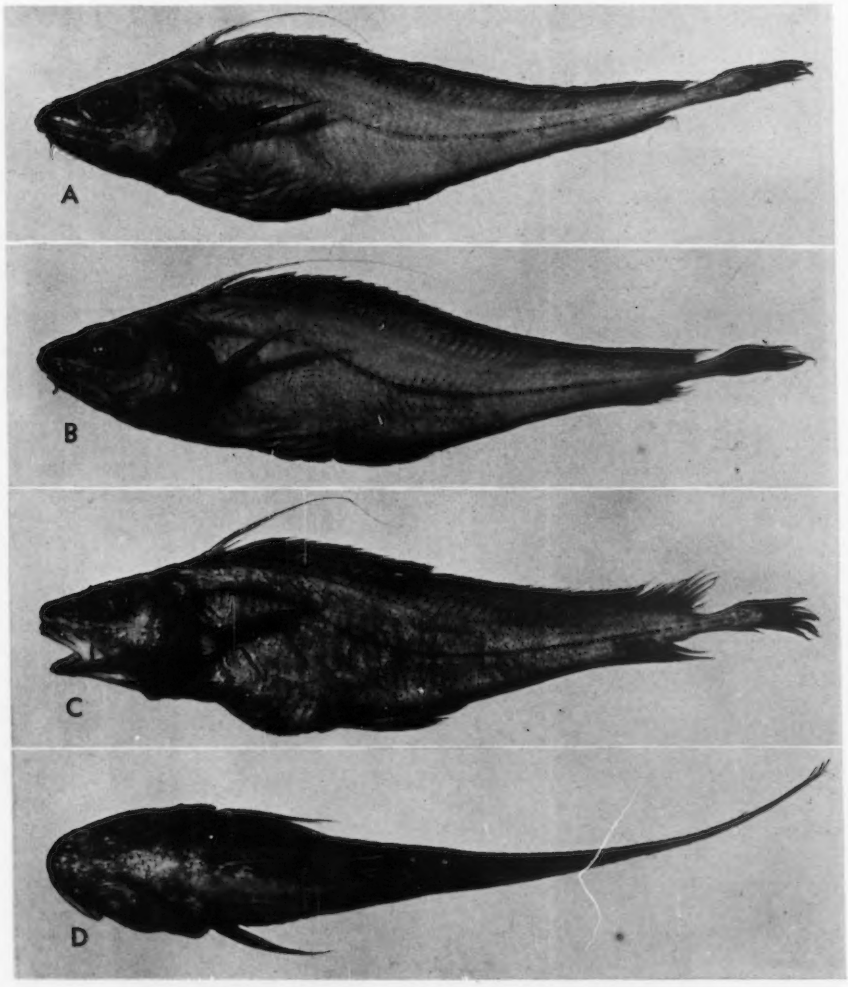

FIG. 1. Lateral views A–C, and dorsal view D, of *Lepidion eques* from Funk Island Bank, northwest Atlantic: A, 264 mm SL, male, and B, 277 mm SL, male, Table 1, No. 13; C and D, 287 mm SL, male, Table 1, No. 11.

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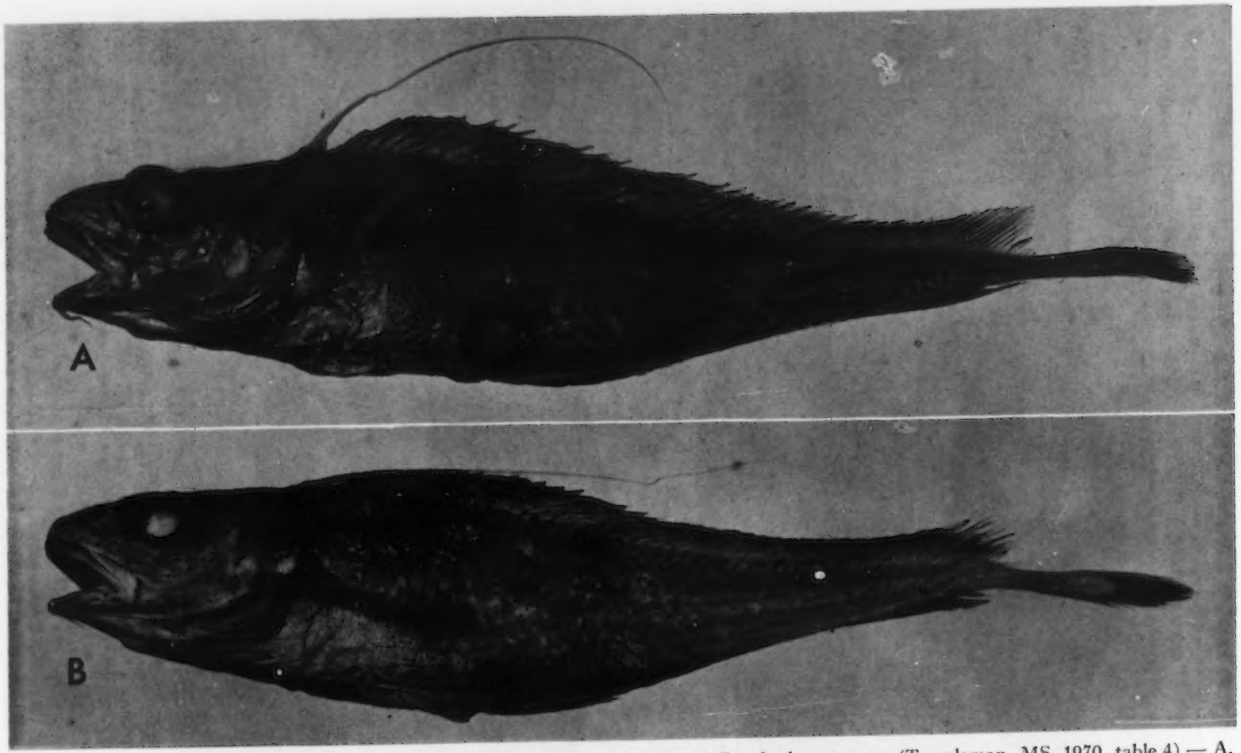

FIG. 2. Lateral views of *Lepidion eques* from the northeast Atlantic — two of Günther's syntypes: (Templeman, MS, 1970, table 4) — A, 235 mm SL, male, No. 9; B, 248 mm SL, male, No. 11.

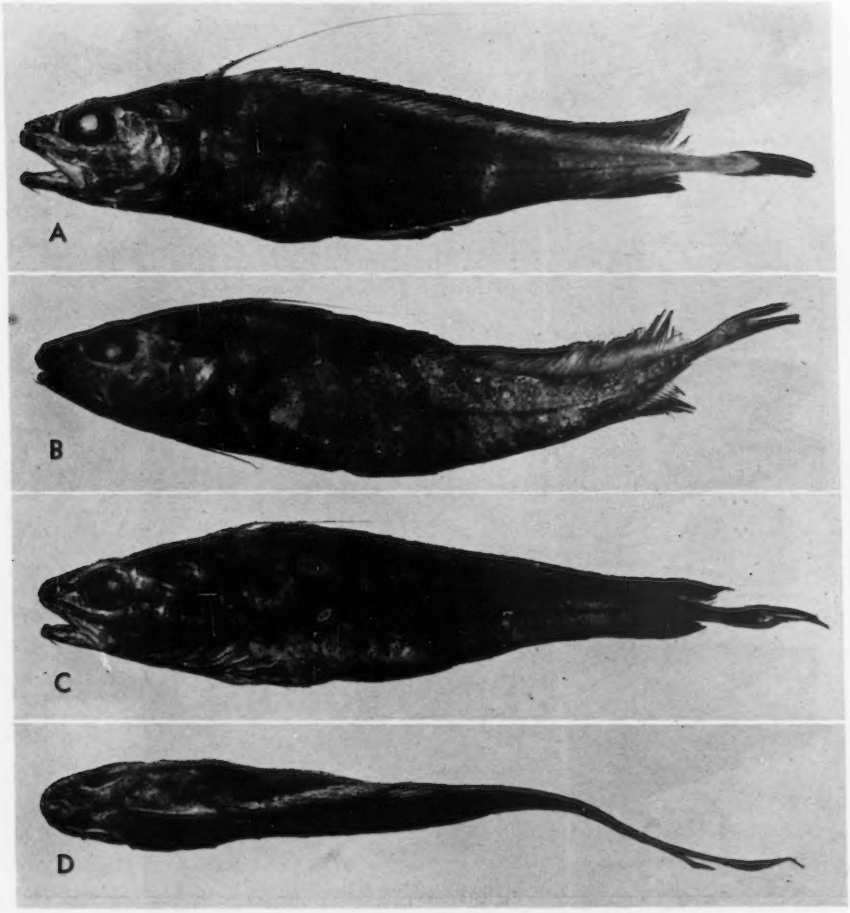

FIG. 3. Lateral views A–C, and dorsal view D, of *Lepidion lepidion* from Mediterranean: (Templeman, MS, 1970, table 5) — A, 221 mm SL, mature male, No. 3; B, 247 mm SL, male, No. 10; C, 256 mm SL, male, No. 12.

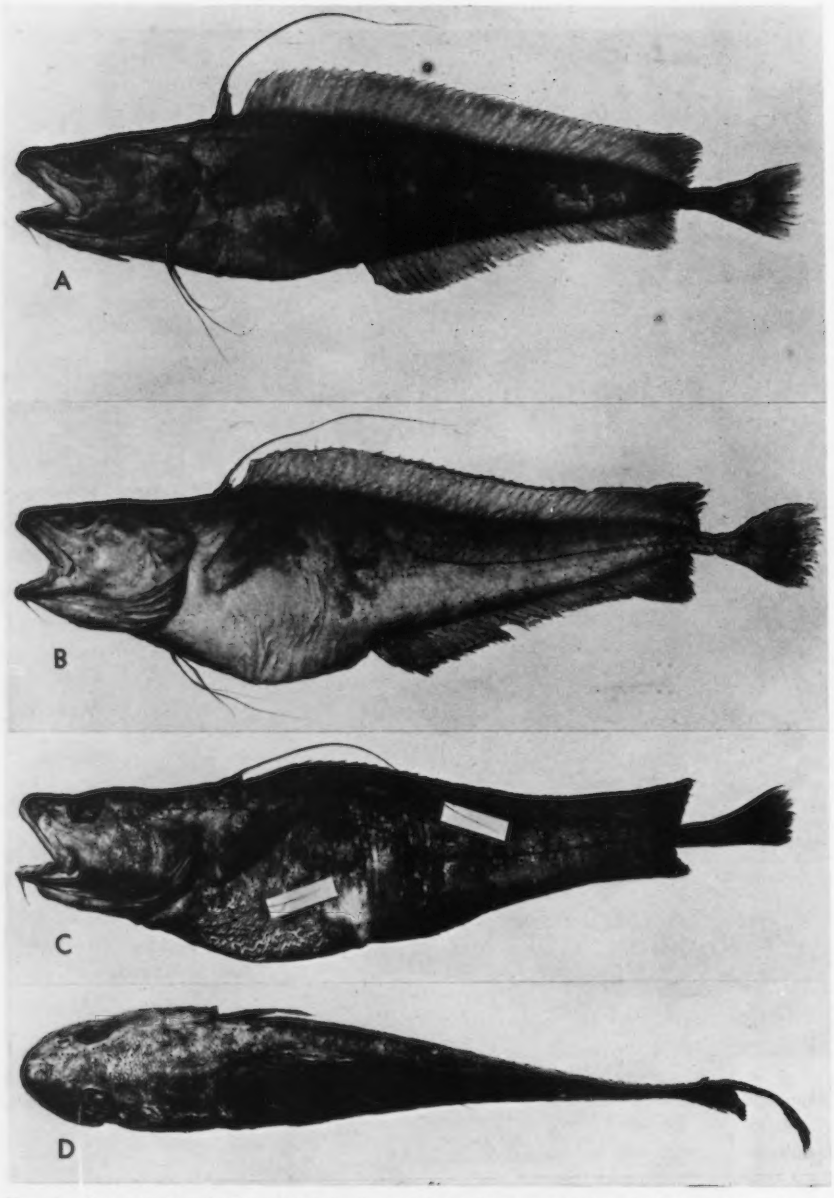

FIG. 4. Lateral views A–C, and dorsal view D, of *Lepidion guentheri* from Madeira: (Templeman, MS, 1970, table 6) — A, 413 mm SL, immature female, No. 2; B, 483 mm SL, male, No. 6; C and D, 536 mm SL, immature female, No. 7.

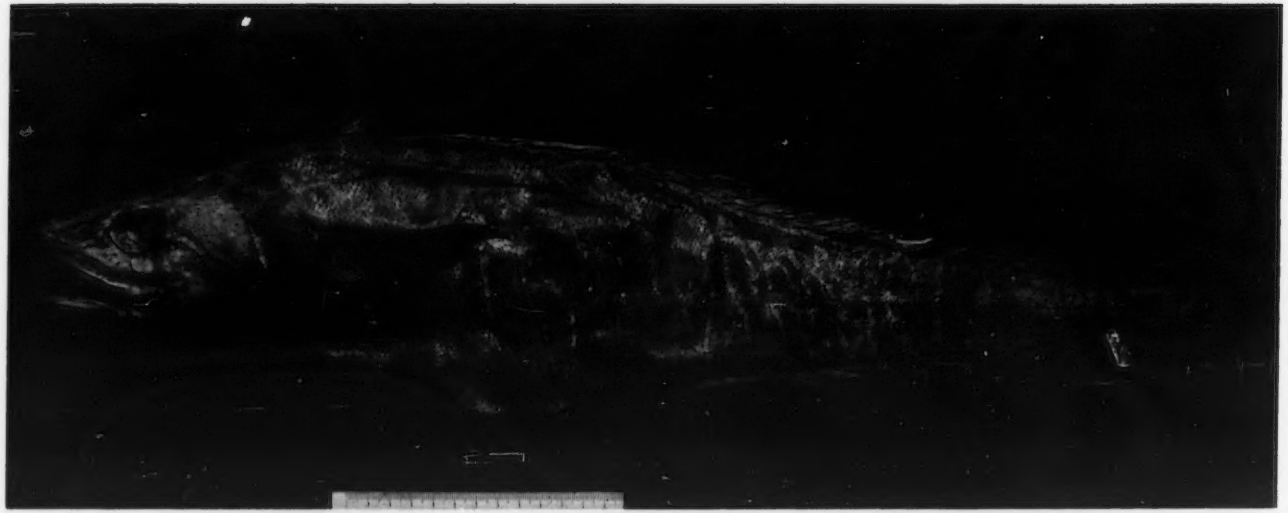

FIG. 5. Lateral view of lectotype of *Lepidion guentheri* from Madeira, 554 mm SL, immature female, Tables 2 and 4, No. b, British Museum (Natural History) 1862.4.22.9. (Photograph by the British Museum (Natural History).)

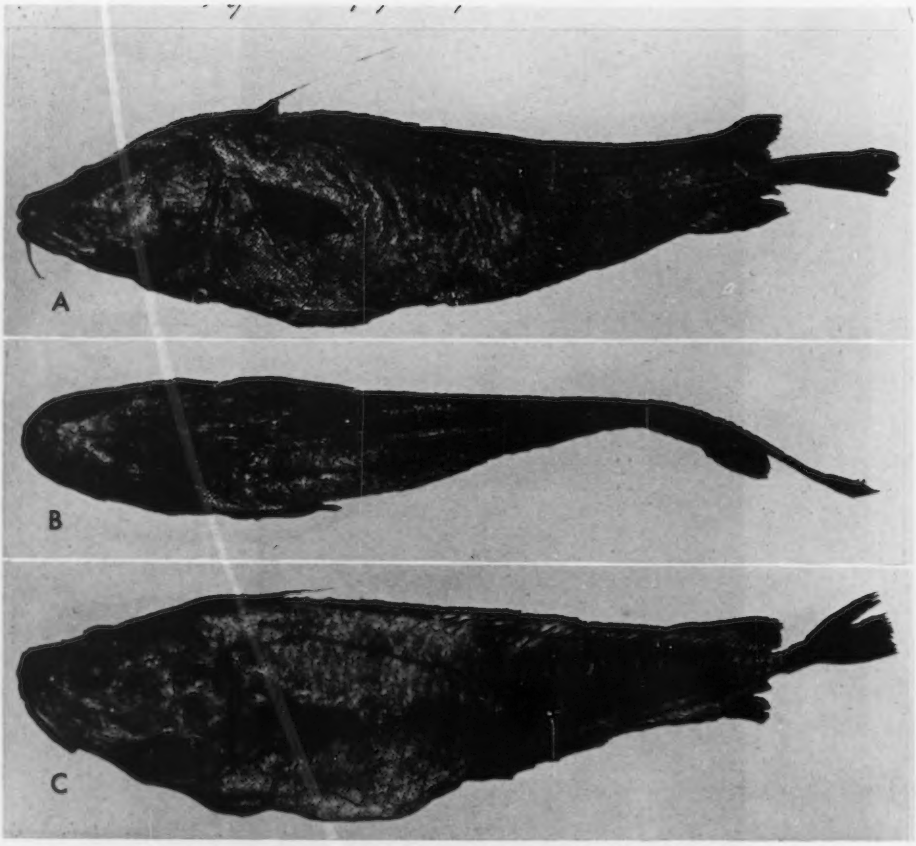

FIG. 6. Lateral views A and C, and dorsal view B, of mature female *Lepidion ?schmidti* from the mouth of the Bay of Biscay: A and B, 1045 mm SL; C, 1127 mm SL; Tables 2 and 4. (Photographs by the British Museum (Natural History).)

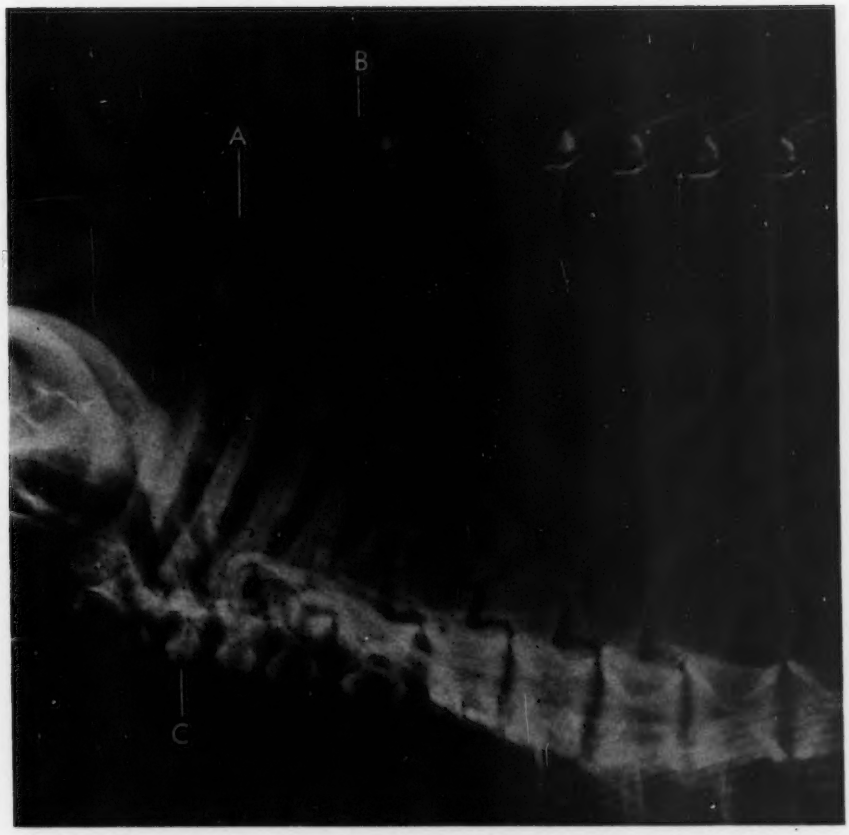

FIG. 7. Radiograph of lateral view of anterodorsal portion of *Lepidion lepidion* from the western Mediterranean, SL 263 mm (Templeman, MS, 1970, table 5, No. 13): A, first neural spine; B, tiny spine or ray in front of the four main first dorsal rays (1+4); C, second centrum of vertebral column, the smallest centrum of the anterior region.

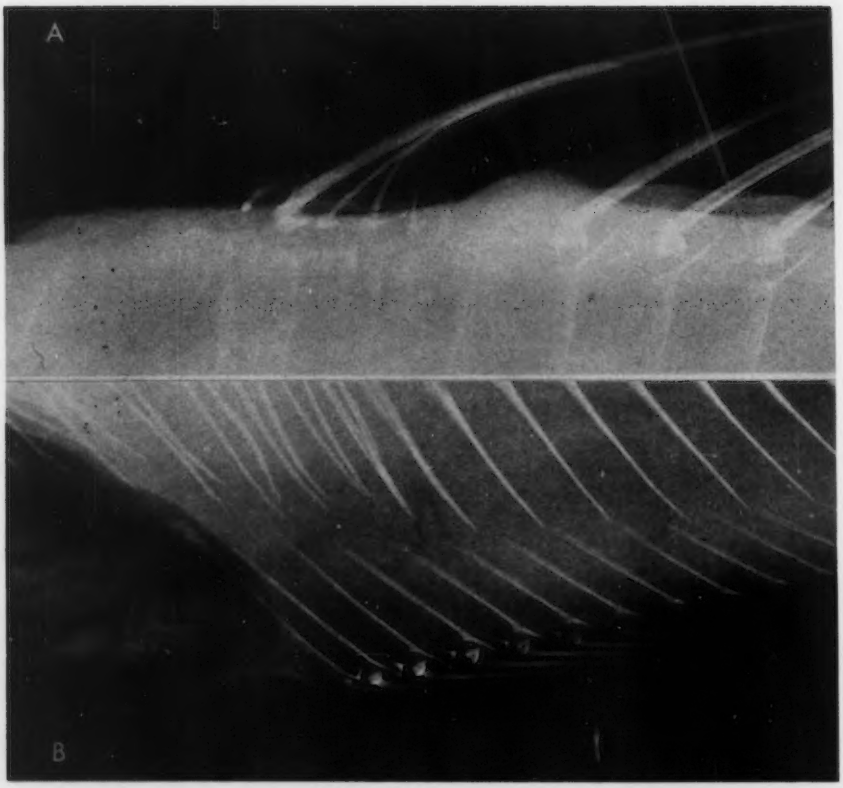

FIG. 8. Radiographs of: A, lateral view first dorsal region of *Lepidion guentheri* from Madeira, SL 536 mm (Templeman, MS, 1970, table 6, No. 7), to show 1+5 first dorsal rays; B, lateral view of anterior region of anal of *Lepidion lepidion* from Messina, SL 254 mm (Templeman, MS, 1970, table 5, No. 11).

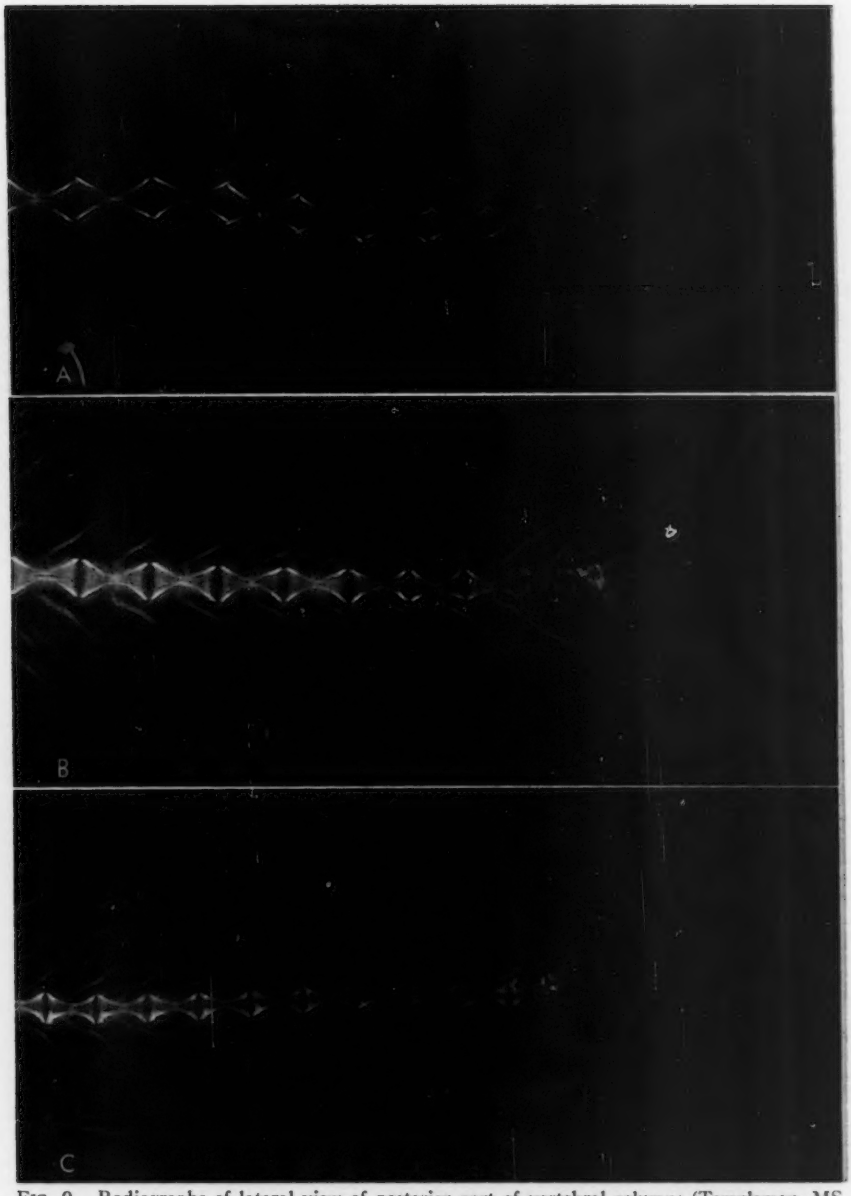

FIG. 9. Radiographs of lateral view of posterior part of vertebral column: (Templeman, MS, 1970, table 6) — A, *Lepidion guentheri*, SL 474 mm, No. 5, normal vertebral column; B, *L. guentheri*, SL 413 mm, No. 2, fourth and third vertebrae from posterior end abnormally shaped but not fused (note also that the two posterior dorsal rays are close together); C, *L. lepidion*, SL 229 mm (Templeman, MS, 1970, table 5, No. 5) (note hypural complex and procurrent rays of caudal are missing, indicating injury and regeneration of caudal at an early stage.

along the body, and sometimes the most posterior dorsal ray, which may be very slender, if it is not closely observed under the microscope. The two long pelvic rays, of which the second is the longer, are closely attached except at their posterior filaments and have evidently been counted as one by some authors, including Günther (1862, 1887) and Vinciguerra (1883). The third pelvic ray is fairly long, close to the second, and is easily missed.

DESIGNATION OF LECTOTYPES

There is a problem concerning the type of *L. guentheri*. The species was described by Johnson (1862) from three specimens taken at Madeira, and by Günther (1862) from a specimen presented by J. Y. Johnson and bearing the British Museum register number 1862.4.22.9. When I saw this specimen in October 1966 the name was recorded on the bottle label as *Haloporphyrus lepidion* from Madeira but in October 1968 the bottle name had been changed to *Lepidion guentheri*. The specimen presented to the British Museum was apparently Johnson's specimen "A" (Tables 2, 4) to which it is nearest in

TABLE 2. Meristic and other number characteristics and related standard lengths of lectotypes of *Lepidion eques* (Günther)[a] and *L. guentheri* (Giglioli)[b] and of the two giant specimens of *Lepidion* sp. (*L. ?schmidti*) of Forster (1968) taken in 1966 and 1967. (Measurements and counts are by Templeman.)

Specimen	SL (*mm*)	Rays						Vertebrae[d]	Gill rakers 1st arch[e]	Pyloric caeca
		1st dorsal	2nd dorsal[c]	Anal[c]	Pectoral	Pelvic	Branchiostegal			
L. eques[a]	271	1+4	57	52	23	8	7	62	5+13	10
L. guentheri[b]	554	1+5	55	50	21	7	7	58	5+17	15
Lepidion sp. 1966	1127	1+5	48	40	24	7	7	54	?+12	–
1967	1045	1+5	47	41[f]	23	7	7	56	6+14	16

[a]British Museum (Natural History), *Haloporphyrus eques* (types), Faroe Channel, *Knight Errant*, 1887. 12.9.60.

[b]British Museum (Natural History), *Haloporphyrus lepidion* (now *Lepidion guentheri*), Madeira, 1862. 4.22.9, presented by J. Y. Johnson.

[c]Last two rays separate and counted separately.

[d]Including urostylar half-vertebra + hypural as one vertebra.

[e]Rudimentary rakers included.

[f]There is additionally a small piece of bone or broken ray anterior to the first anal ray. This does not have the base of a typical ray, is not represented by a pterygiophore, and was not counted as a ray.

length. Johnson said that three specimens were taken at Madeira, and he described all three of them. On the whole, Johnson gave a more complete description than Günther. Since both authors named the fish *H. lepidion* Risso, neither of them designated a type specimen.

When Giglioli (1880) named Günther's and Johnson's fish *H. güntheri* he did not examine any fish of this species, depending on Günther's description

for differences from Risso's *H. lepidion*. Giglioli says that his new species, *H. güntheri*, has a much smaller eye and a much longer snout and barbel than *H. lepidion*. Günther (1862) mentions the smallness of the eye in relation to length of head and Johnson (1862) the longer snout in relation to the eye. The snout itself is not necessarily longer in *L. guentheri* than in *L. lepidion* but it is much longer in relation to the eye diameter. Similarly the main difference between the length of the barbel of the two species, although the length in *L. guentheri* averages longer, is its greater length in *L. guentheri* in relation to the eye.

Günther's description of *H. lepidion* and his substitution of *Haloporphyrus* as a genus name for *Lepidion* appeared on p. 358–359 of Vol. 4, Catalogue of the fishes in the British Museum, 1862. This volume was published not earlier than November 8, 1862, the evidence for this in a letter from Mr Alwyne Wheeler of the British Museum (Natural History) being as follows: November 8, 1862, was the date of the Trustees' meeting at which the selling price of Volume 4 was set. The volume could not therefore have been published before that date, although it probably was published very soon afterwards. Johnson's paper, however, on this species bears the date of September 1862, and Mr Wheeler is of the opinion that there is not much doubt that Johnson's paper was the earlier of the two. In using the new genus *Haloporphyrus*, Johnson refers it to "Günther, MS." He also uses *Haloporphyrus lepidion*, Günther. Hence he must have received information from Günther or seen his manuscript on *H. lepidion*. Johnson's description and measurements, however, are not similar to Günther's and are more extensive. He must also have carried out his measurements and other observations on Günther's specimen before he sent it to Günther. He also precedes Günther in publication and hence his naming of the genus *Haloporphyrus* and description of the species *H. lepidion* (*H. guentheri*) have precedence over Günther's.

The matter of genus naming is only of historical importance for this paper since I have reverted to the genus name *Lepidion*. As Giglioli based his description on Günther's (who used one of Johnson's previously described specimens), Johnson's three specimens are therefore syntypes of which the only known specimen today is the British Museum specimen 1862.4.22.9 above and Fig. 5 (described by Johnson (1862), Günther (1862), and by me in Tables 2, 4), which I here designate as the lectotype of *L. guentheri*.

Lepidion eques (Fig. 1, 2) was described by Günther (1887) as *H. eques* from specimens taken by the *Knight Errant* in the Faroe Channel. Almost all the subsequent captures of *Lepidion* from the North Atlantic outside the Mediterranean (apart from *L. guentheri*) have been referred to this species (Table 1; Templeman, MS, 1970, table 1).

Günther (1887) did not designate a type specimen of *L. eques*, but in the British Museum (Natural History) under "No. 25460 1887.12.9.58–67, *Haloporphyrus eques* (types) Faroe Channel, *Knight Errant*" are 11 specimens of *L. eques* that are evidently Günther's syntypes of this species. These are specimens caught by the vessel *Knight Errant* in the Faroe Channel in 1880

and 1882. These specimens have all been reexamined in detail by this author for meristic characters (Templeman, MS, 1970, table 2, specimens 1*a–k*) and for morphometric characteristics (Templeman, MS, 1970, table 4, specimens 2, 3, 5, 7, 9–12). From them I have selected as the lectotype specimen No. *a* of table 2 and table 4, which is No. 60 in the above numbering system of the British Museum. This specimen is extreme in some respects but so are all the suitable specimens of moderate size from which it was thought desirable to select the lectotype. The other specimens of this group are the paralectotypes of *L. eques*.

LEPIDION EQUES FROM THE NORTHWEST AND THE NORTHEAST ATLANTIC

Meristic Characters

The groups compared by the *t*-test are from the northwest Atlantic (1953–67), from the Faroe Channel (1880–82), and from west of Ireland (1905, 1961). For all meristic characters there is considerable and usually almost complete overlapping among *L. eques* from these areas (Table 3). For second dorsal fin-ray numbers there is a significant difference ($P = 0.05$) between the means of the Newfoundland and west of Ireland groups but not between those for Newfoundland and the Faroes nor the Faroes and west of Ireland. For anal fin rays, pectoral rays, gill rakers on the lower limb of the first branchial arch (subsequently called gill rakers or gill-raker number), and number of pyloric caeca, there are no significant differences between the means for the above areas. The means of vertebral numbers for the northwest Atlantic and for the Faroes and west of Ireland combined differ ($P < 0.05$).

Morphometric Characters

In the northwest Atlantic fish, the orbit is usually smaller and the interorbital width also tends to be greater than in fish from the northeast Atlantic, especially when compared with the head length (Fig. 10, 11). Also, for the specimens from the northwest Atlantic, the interorbital width, snout length, and postorbital length in relation to the horizontal diameter of the orbit are usually greater than in those from the northeast Atlantic (Fig. 14). However, in all of these the ranges overlap somewhat at the same fish lengths. The first long ray of the first dorsal is relatively shorter and the least height of the caudal peduncle relatively greater in the northwest Atlantic specimens but the ranges overlap (Fig. 12, 13). It is not certain how much these last two differences can be depended upon because it is usually difficult to be certain whether or not the filamentous tip of the long dorsal ray has been broken. Also, with regard to the differences in least height of the caudal peduncle, see later discussion.

For the remaining morphometric characters (Fig. 10–14; Templeman, MS, 1970, tables 3, 4), the values from both sides of the Atlantic for the same length groups are well intermingled.

TABLE 3. Frequencies of various numbers of fin rays, vertebrae, gill rakers, and pyloric caeca in North Atlantic species of *Lepidion*.[a]

Species	Locality	No. second dorsal fin rays										No. fish	Mean	SD	SE
		47	48	53	54	55	56	57	58	59	60				
L. eques	NW Atlantic	–	–	–	–	1	2	4	4	2	2	15	57.67	1.45	0.37
"	Faroe Chan.	–	–	–	–	1	1	4	2	2	1	11	57.55	1.44	0.43
"	W of Ireland	–	–	–	–	–	5	5	2	–	–	12	56.75	0.75	0.22
"	Total all areas[b]	–	–	–	–	2	8	13	9	4	3	39	57.36	1.29	0.21
L. lepidion	Mediterranean	–	–	–	5	5	0	1	1	1	–	13	55.31	1.65	0.46
L. guentheri	E Atlantic	–	–	–	1	1	4	1	1	–	–	8	56.00	1.20	0.42
L. ?schmidti	Bay of Biscay	1	1	–	–	–	–	–	–	–	–	2	47.50	–	–
		No. anal fin rays													
		40	41	47	48	49	50	51	52	53	54				
L. eques	NW Atlantic	–	–	–	–	–	–	1	5	7	1	14	52.57	0.76	0.20
"	Faroe Chan.	–	–	–	–	–	2	1	2	2	4	11	52.45	1.57	0.47
"	W of Ireland	–	–	–	–	–	1	1	4	6	–	12	52.25	0.97	0.28
"	Total all areas[b]	–	–	–	–	–	3	3	12	15	5	38	52.42	1.08	0.18
L. lepidion	Mediterranean	–	–	–	2	5	3	3	–	–	–	13	49.54	1.05	0.29
L. guentheri	E Atlantic	–	–	–	–	1	3	1	2	1	–	8	50.87	1.36	0.48
L. ?schmidti	Bay of Biscay	1	1	–	–	–	–	–	–	–	–	2	40.50	–	–
		No. vertebrae (including hypural)													
		54	55	56	57	58	59	60	61	62					
L. eques	NW Atlantic	–	–	–	–	1	0	7	5	2		15	60.47	0.99	0.26
"	Faroe Chan.	–	–	–	1	0	3	4	1	1		10	59.70	1.34	0.42
"	W of Ireland	–	–	–	–	–	6	3	3	–		12	59.75	0.87	0.25
"	Total NE Atlantic	–	–	–	1	0	9	7	4	1		22	59.73	1.03	0.22
"	Total all areas	–	–	–	1	1	9	14	10	3		38	60.05	1.09	0.18
L. lepidion	Mediterranean	–	–	3	3	5	–	–	–	–		11	57.18	0.87	0.26
L. guentheri	E Atlantic	–	–	–	3	4	1	–	–	–		8	57.75	0.71	0.25
L. ?schmidti	Bay of Biscay	1	0	1	–	–	–	–	–	–		2	55.00	–	–

No. pectoral fin rays

		19	20	21	22	23	24	25				
L. eques	NW Atlantic	–	–	1	3	8	2	1	15	22.93	0.96	0.25
,,	Faroe Chan.	–	–	–	3	6	1	1	11	23.00	0.89	0.27
,,	W of Ireland	–	–	–	6	3	3	–	12	22.75	0.87	0.25
,,	Total all areas[b]	–	–	1	12	17	7	2	39	22.92	0.84	0.13
L. lepidion	Mediterranean	–	–	1	7	4	1	–	13	22.38	0.77	0.21
L. guentheri	E Atlantic	1	1	4	2	–	–	–	8	20.88	0.99	0.35
L. ?schmidti	Bay of Biscay	–	–	–	–	1	1	–	2	23.50	–	–

No. gill rakers on lower limb first branchial arch

		12	13	14	15	16	17	18				
L. eques	NW Atlantic	–	–	5	8	2	–	–	15	14.80	0.68	0.18
,,	Faroe Chan.	–	2	2	5	2	–	–	11	14.64	1.03	0.31
,,	W of Ireland	–	1	4	4	3	–	–	12	14.75	0.97	0.28
,,	Total all areas[b]	–	3	12	17	7	–	–	39	14.72	0.86	0.14
L. lepidion	Mediterranean	–	1	3	5	3	0	1	13	15.08	1.26	0.35
L. guentheri	E Atlantic	–	–	–	–	5	2	1	8	16.50	0.76	0.27
L. ?schmidti	Bay of Biscay	1	0	1	–	–	–	–	2	13.00	–	–

No. pyloric caeca

		8	9	10	11	12	13	14	15	16	17				
L. eques	NW Atlantic	–	7	3	4	1	–	–	–	–	–	15	9.93	1.03	0.27
,,	Faroe Chan.	–	1	5	3	2	–	–	–	–	–	11	10.55	0.93	0.28
,,	W of Ireland	1	1	2	6	0	1	–	–	–	–	11	10.55	1.29	0.39
,,	Faroe Chan. + W of Ireland	1	2	7	9	2	1	–	–	–	–	22	10.55	1.10	0.23
,,	Total all areas[b]	1	9	10	13	4	1	–	–	–	–	38	10.34	1.12	0.18
L. lepidion	Mediterranean	1	4	4	–	–	–	–	–	–	–	9	9.33	0.71	0.24
L. guentheri	E Atlantic[c]	–	–	–	–	–	–	–	5	1	4	10	15.90	0.99	0.31
L. ?schmidti	Bay of Biscay	–	–	–	–	–	–	–	–	1	–	1	16	–	–

[a]My counts only unless otherwise noted.
[b]Total includes a fish from W of Iceland.
[c]Two counts included from Maul (1952) for fish not examined by me.

TABLE 4. Morphometric characteristics of the lectotype of *Lepidion eques* (Günther),[a] *L. guentheri* (Giglioli),[b] and of the two giant specimens of *Lepidion* sp. (*L. ?schmidti*) taken in 1966 and 1967 (Forster, 1968). (Measurements are by Templeman.)

Item no.	Body part	Lectotype[a] of *L. eques*	Lectotype[b] of *L. guentheri*	*Lepidion* sp. (*L. ?schmidti*) Specimen 1	*Lepidion* sp. (*L. ?schmidti*) Specimen 2
1	Total length (greatest) (*mm*)	293+	605	1150	1230
2	Standard length (*mm*)[c]	271	554	1045	1127
3	Head: length (*mm*)[d]	60.5	133.3	275	287
4	Head: length	22.3	24.1	26.3	25.5
5	Orbit: horizontal diam	7.6	4.5	5.3	ca. 4.6
6	Interorbital width[e]	5.1	5.1	6.5	6.6
7	Snout: length	6.7	7.3	8.1	8.0
8	Postorbital length	9.0	12.0	13.3	13.8
9	Barbel: length	3.7	5.3	8.6	6.6
10	Maxillary extension backward relative to eye[f]	1/2	2/3	3/5	1/2
11	1st dorsal: 1st long ray, length	51.7	36.3	39.7	[g]
12	Pectoral: length	16.5	15.0	16.1	14.0
13	Pelvic: length to tip longest ray	13.5	24.3	18.6	16.3
14	Body: greatest height	20.7	ca. 25.2	ca. 23.9	ca. 27.5
15	Caudal peduncle: least height	2.0	ca. 3.2	3.1	ca. 3.1
16	Snout to anterior base 1st ray 1st dorsal	23.6	26.9	29.9	28.6
17	Snout to midanus	41.0	47.7	50.7	52.3
18	Snout to anterior insertion anal	43.9	–	53.1	54.5
19	Outer base pelvic to anterior insertion anal	22.5	ca. 29.4	30.5	32.3
20	Posterior insertion 2nd dorsal to beginning upper procurrent rays caudal	5.4	4.9	3.9	3.7
21	Posterior base last anal ray anterior to posterior base last ray 2nd dorsal[h]	2.1	1.4	0.5	1.4
22	Posterior base last ray 2nd dorsal to base midcaudal	9.5	8.4	8.2	7.5
23	Length longest pyloric caecum	10.0	20.4	9.6	–
24	Midanus below what ray 2nd dorsal	10–11	–	10–11	10–11
25	Origin anal below what ray 2nd dorsal	12–13	12–13	12	12
26	Anterior end steepest slope lateral line posterior to anterior insertion anal[h]	–3.3	1.9[i]	–	–
27	Posterior end steepest slope lateral line posterior to anterior insertion anal[h]	5.2	8.9[i]	–	–
28	Point where lateral line first is equidistant from dorsal and anal fin-ray bases posterior to anterior insertion anal	8.1	–	–	–
29	Sex and maturity	♂	♀ immature	♀ mature	♀ mature

[a], [b] As in Table 2.
[c] Snout to end hypural.
[d] Snout to end of bony operculum (to end of opercular spine if this is the greatest measurement). The postorbital is measured similarly posteriorly.
[e] Least fleshy width.
[f] With mouth closed.
[g] Unsuitable for this measurement.
[h] Shortest distance between parallel transverse lines.
[i] From lateral view photograph.

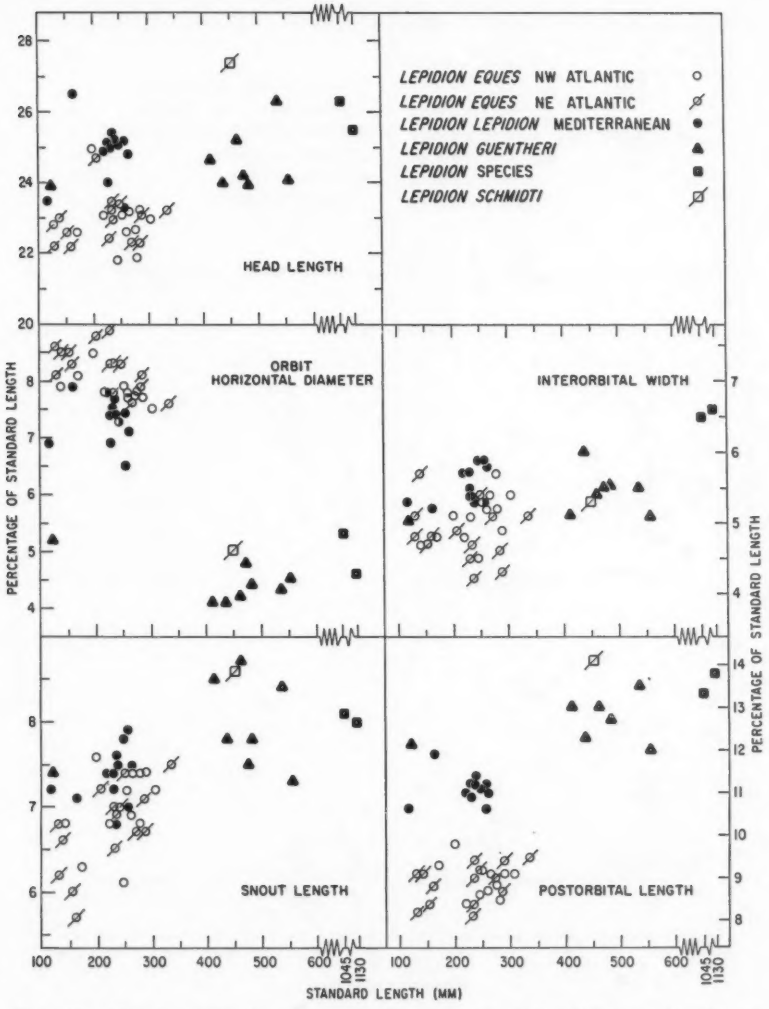

FIG. 10. Morphometric characteristics of the species of *Lepidion* from the North Atlantic. (Data from *L. schmidti* have been added for comparison.)

DISCUSSION AND CONCLUSIONS

MERISTIC CHARACTERS

For the few significant differences in means of meristic characters between the western and eastern Atlantic *L. eques*, most of the fish from the northeast were from a considerably earlier period than those from the northwest Atlantic. The west of Ireland group is made up of components from the years 1905 and 1961. There is no apparent difference in the meristic characters of these

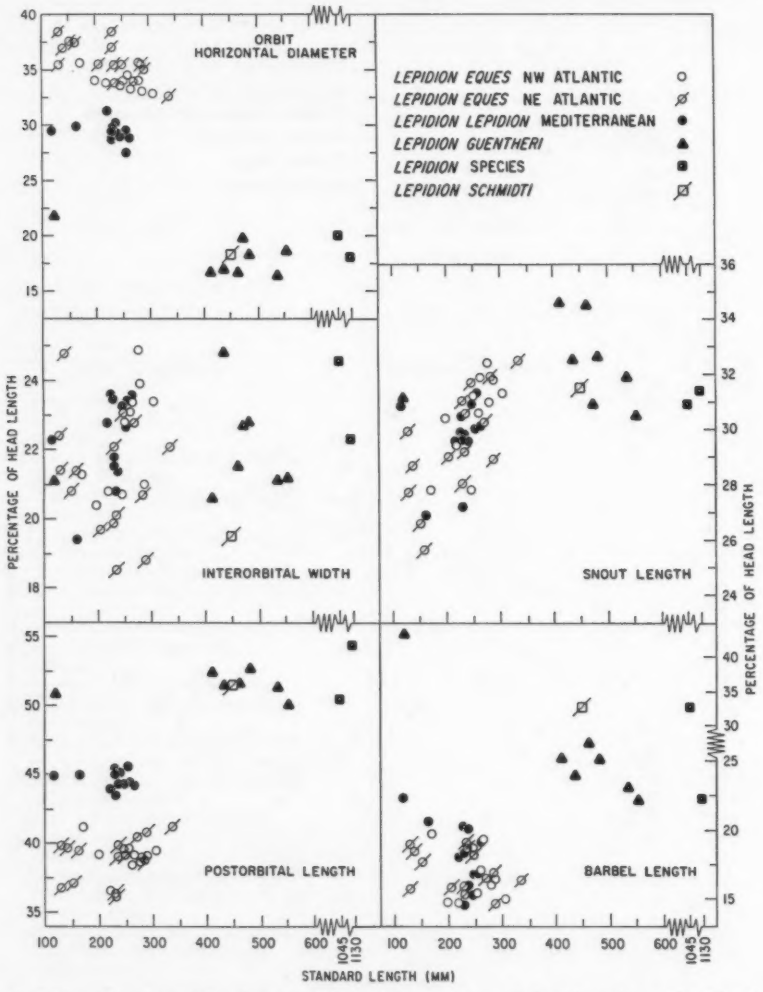

FIG. 11. Morphometric characteristics of the species of *Lepidion* from the North Atlantic. (Data from *L. schmidti* have been added for comparison.)

two components except that vertebral numbers may be slightly lower in the earlier period. This is opposite to the expected result in that the earlier period should have had lower temperatures.

The meristic characters that one might expect to be most affected by temperature — numbers of rays in the second dorsal and anal fins and of vertebrae — are all higher or tend to be higher in the northwest than in the northeast Atlantic, the values for the two fins and also for the total of all three characters being lowest for fish from west of Ireland. The temperatures

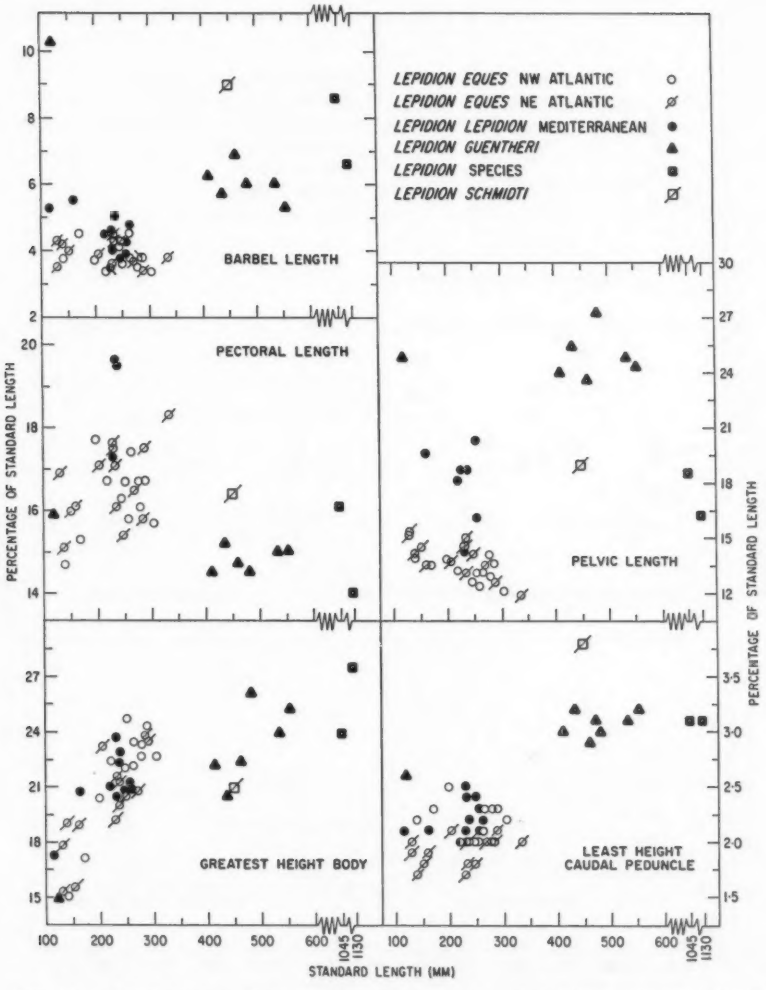

FIG. 12. Morphometric characteristics of the species of *Lepidion* from the North Atlantic. (Data from *L. schmidti* have been added for comparison.)

of the deep water inhabited by *L. eques* are much higher in the northeast than in the northwest Atlantic (Table 1; Templeman, MS, 1970, table 1) and the differences would probably be narrowed little by climatic changes from 1880 to 1967, the period during which the specimens studied were taken. It is not known at what depths the eggs and early larvae occur when the meristic numbers are being determined. However, judging from halibut, the Greenland halibut (Jensen, 1935), and the argentine *Argentina silus* (Schmidt, 1906), which spawn deeply and in which the eggs are bathypelagic and remain deeply

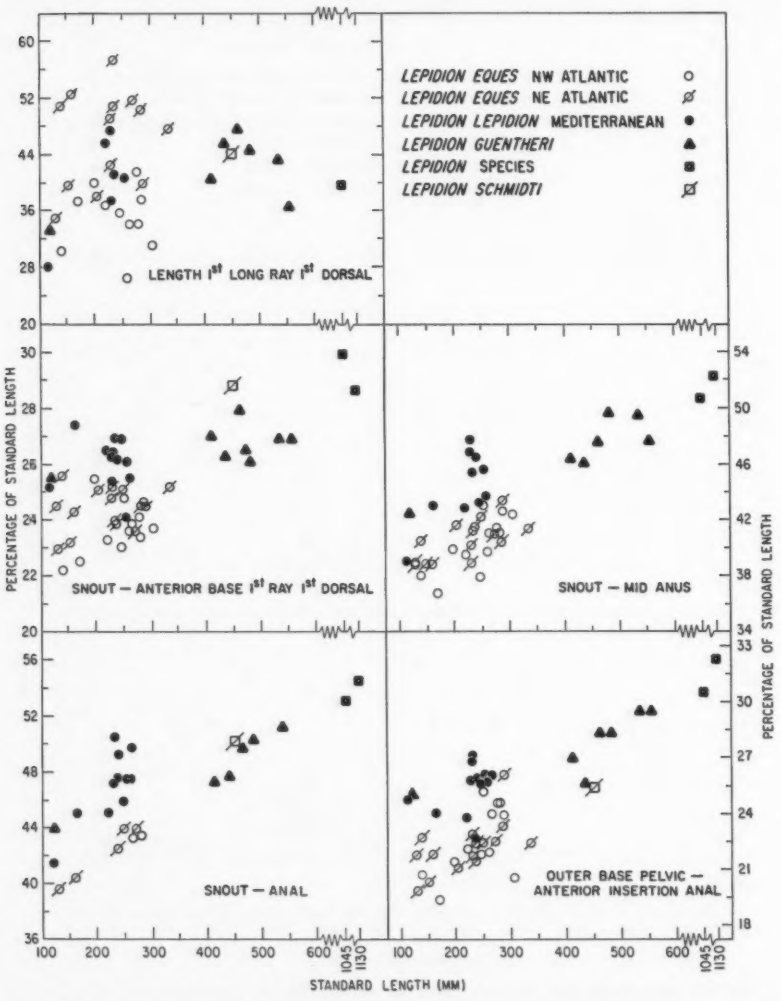
LEPIDION EQUES NW ATLANTIC
LEPIDION EQUES NE ATLANTIC
LEPIDION LEPIDION MEDITERRANEAN
LEPIDION GUENTHERI
LEPIDION SPECIES
LEPIDION SCHMIDTI
LENGTH 1ST LONG RAY 1ST DORSAL
SNOUT — ANTERIOR BASE 1ST RAY 1ST DORSAL
SNOUT — MID ANUS
SNOUT — ANAL
OUTER BASE PELVIC — ANTERIOR INSERTION ANAL
PERCENTAGE OF STANDARD LENGTH
PERCENTAGE OF STANDARD LENGTH
STANDARD LENGTH (MM)

FIG. 13. Morphometric characteristics of the species of *Lepidion* from the North Atlantic. (Data from *L. schmidti* have been added for comparison.)

distributed, the eggs and early larvae of *L. eques* probably are distributed deeply enough for the deepwater temperature differences between the two areas to have an effect.

Actually, we have no evidence regarding the spawning depths and areas of *Lepidion* in the northwest Atlantic, and for the considerably higher deepwater temperatures in the northeast than in the northwest Atlantic greater meristic differences than those noted above would be expected. There is,

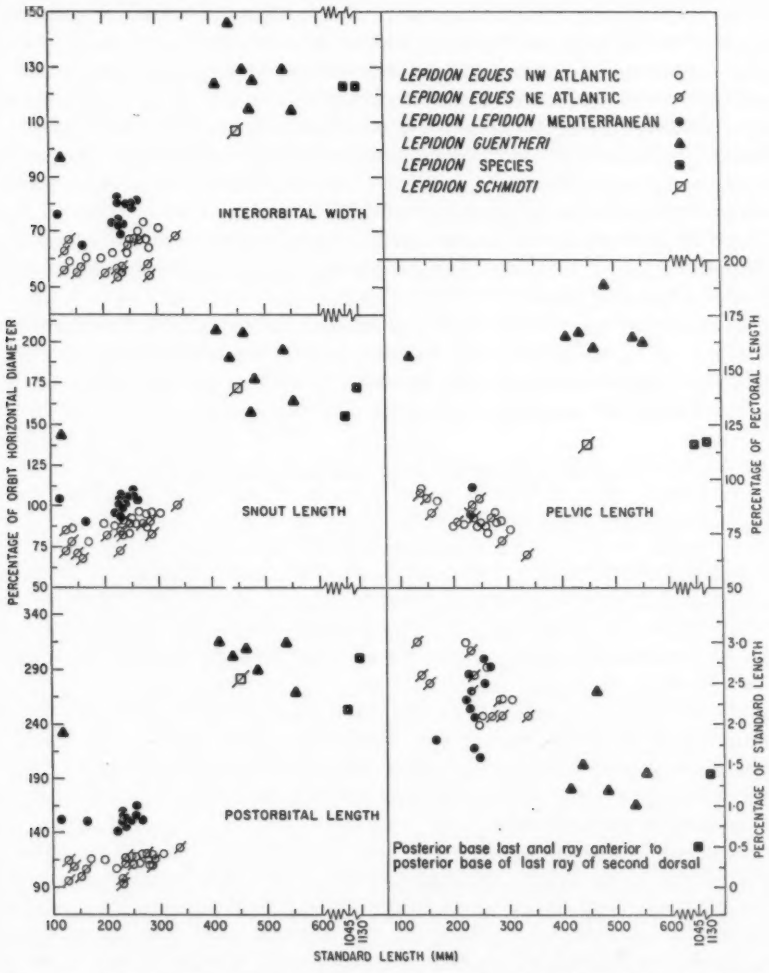

FIG. 14. Morphometric characteristics of the species of *Lepidion* from the North Atlantic. (Data from *L. schmidti* have been added for comparison.)

however, a large population southwest of Iceland and here deepwater temperatures are intermediate between those of the northwest and of the northeast Atlantic. The northwest Atlantic specimens very likely came from the southwestern Icelandic area by way of west Greenland.

MORPHOMETRIC CHARACTERS

For the tendency of the first long ray of the first dorsal to be relatively shorter (and the least height of the caudal peduncle relatively greater) in

L. eques from the northwest than from the northeast Atlantic, I have no explanation as more study of this character against depth and sex is required in the north-central or northeast Atlantic where the species is more numerous. These fish were caught in bottom trawls and it is not often certain whether or not the fine filamental extension of this first long ray has been broken. The difference in least height of the caudal peduncle in specimens from the two areas should be disregarded for the present. This character was measured for the northwest Atlantic on specimens from 10% formalin after soaking in water. The specimens from the northeast Atlantic were from ethyl alcohol, presumably 70% or higher, and I have noted that shrinkage occurs when formalin specimens are later placed in alcohol.

It is evident from the data presented that the meristic and morphometric characters of *L. eques* from the northwest and northeast Atlantic overlap so much that individuals from the different areas are not separable on these bases and that all belong to the same species.

LEPIDION EQUES AND *LEPIDION LEPIDION*

Meristic Characters

Lepidion lepidion has fewer ($P < 0.001$) second dorsal fin rays, anal fin rays, and vertebrae than *L. eques* (Table 3). There is almost complete overlapping in the ranges for the second dorsal, 54–59 vs. 55–60 (*L. lepidion* first), and appreciably less in those for the anal fin rays and vertebrae, 48–51 vs. 50–54 and 56–58 vs. 57–62.

The number of pyloric caeca for the nine *L. lepidion* examined for this character is in the lower part of the range of *L. eques*, 8–10 vs. 8–13, and the means differ ($P < 0.005$). The shape of the distribution indicates that as many as 11 pyloric caeca may be expected for *L. lepidion* if larger numbers of this species are examined.

The two species differ ($P < 0.05$) in number of pectoral fin rays but not of gill rakers on the lower limb and in both cases the *L. lepidion* range is included in the *L. eques* range except for one high gill-raker count for *L. lepidion*. However, *L. lepidion* may possess on the average more gill rakers than *L. eques* on the upper limb of the first branchial arch, having 12 specimens with six and only 1 specimen with five gill rakers on the upper limb whereas *L. eques* has 22 with six and 17 with five.

Morphometric Characters

In *L. lepidion* the horizontal diameter of the orbit is less than in *L. eques*. The ranges are separate for this orbit diameter in relation to head length and overlap slightly in relation to standard length. The postorbital length is distinctly greater in *L. lepidion*, especially in relation to the head length, and the range is separate from that of *L. eques*. Because of the smaller eye and greater postorbital length, the postorbital length in relation to the horizontal diameter

of the orbit is distinctly greater in *L. lepidion* than in *L. eques* and the values are especially well separated at the same standard lengths (Fig. 10, 11, 14).

The head length, interorbital width, and snout length in relation to the standard length, and the snout length and interorbital width in relation to the horizontal diameter of the orbit, are greater in *L. lepidion* than in *L. eques*. At the same standard lengths there is overlapping, sometimes only slight, with the upper part of the range of the latter (Fig. 10, 14).

The pelvic fins are relatively longer and the distances from snout to midanus, from snout to anterior base of first ray of first dorsal, and from pelvic base to anterior insertion of anal are relatively greater in *L. lepidion* with usually only slight overlapping with the upper part of the range of *L. eques*. The distance from snout to first anal is distinct at the same fish lengths but this distance was measured in only a few *L. eques* (Fig. 12, 13).

The other morphometric characters of the two species are more nearly alike, those for *L. lepidion* being within or overlapping considerably with the ranges for *L. eques* (Fig. 10–14).

In *L. eques* the horizontal diameter of the orbit shows some negative allometry and the snout length some positive allometry with increase in fish length. The greatest body height, the distances from snout to midanus and from snout to first anal, and the distance from outer base of pelvic to anterior insertion of anal show a strong positive allometry with increasing fish length. It has been noted, as would be expected, that increased contents of the body cavity, such as when a female possesses a large ovary, tend to increase the relative body height. The pectoral length shows some positive allometry but the pelvic length and the pelvic length in relation to the pectoral length show strong negative allometry with increasing fish length.

Presumably similar trends occur in *L. lepidion*, but the lengths of most of the *L. lepidion* measured cover too small a range for the allometric trends to appear as often or as distinctly as in the *L. eques* data. There were many more measurements of *L. eques* than of *L. lepidion* within the length range of the latter, and the comparisons between them are for approximately the same ranges of standard lengths. Therefore, the evident positive or negative allometry in many morphometric characters of *L. eques* does not interfere with comparisons with *L. lepidion*.

Discussion and Conclusions

Meristic characters

There are significant differences between means of many meristic characters in *L. eques* and *L. lepidion* but enough overlapping in all these that individuals cannot be separated by meristic methods unless lateral line scale numbers are different and can be used. For most specimens this method is not available.

The considerably lower meristic numbers, except for gill rakers, for *L. lepidion* of the Mediterranean than for *L. eques* of the North Atlantic are to be expected and are customary in Mediterranean compared with northern North Atlantic species because of the higher temperatures of the Mediterranean.

The higher salinities of the Mediterranean may possibly have some influence on meristic number, but if they have, it is uncertain whether they act to increase or decrease the effect of temperature. For *Gasterosteus aculeatus*, Heuts (1949) demonstrated meristic numbers increasing with increasing salinity at some temperatures and decreasing with increasing salinity at other temperatures. However, for cod (Schmidt, 1930) it is apparent that water temperatures rather than salinities have the controlling influence on vertebral number determination in the eastern Baltic.

MORPHOMETRIC CHARACTERS

In the small number of specimens I measured, the smaller horizontal orbit diameter in relation to the head length and especially the relatively larger postorbital length in *L. lepidion* and the consequent greater difference when the postorbital length is expressed as a percentage of the orbit diameter (Fig. 10, 11, 14) separate this species from *L. eques*. The remaining morphometric characters measured overlap in range, although in many cases only slightly or in an occasional individual, but are thus not sufficiently different for species separation.

With a head length usually relatively greater in *L. lepidion* than in *L. eques*, a snout length in terms of the head length approximately similar in both species, and a smaller eye diameter in *L. lepidion*, the postorbital length is relatively greater in *L. lepidion*.

Although in the horizontal diameter of the orbit *L. eques* from the northwest Atlantic is also moderately well separated in most cases from *L. eques* of the northeast Atlantic, the relative postorbital lengths of *L. eques* from these two areas are well intermingled both in terms of head length and of standard length. Hence the longer postorbital in *L. lepidion* may be indicative of a species separation.

GENERAL

Günther (1887) from specimens taken by the *Knight Errant* in the Faroe Channel in 1880 and 1882 described *H. eques* as a new species, his differences between this species and the Mediterranean *H. lepidion* being that the maxillary in *H. lepidion* extended beyond the middle of the orbit and in *H. eques* to nearly below the middle of the eye. Although in his descriptions Günther indicates that the head in *H. lepidion* is 3–3½ times and in *H. eques* 3 times the length of the eye he also states his conviction that *H. eques* can be distinguished at all sizes from *H. lepidion* by its comparatively larger eye and shorter head.

Günther records meristic characters for *H. eques* and *H. lepidion* (in parentheses) as follows: second dorsal 56–62 (52), anal 49–54 (46–48), pelvic 7 (6), scales above the lateral line 15–16 (13–14), lateral line scales 180 (155–160). The maxillary usually, but not always, extends farther posteriorly in relation to the eye in *L. lepidion* than in *L. eques* but, since the snout in relation to the head length is on the average approximately the same in the two species, the more posterior end of maxillary–eye relation in *L. lepidion* is presumably related to its smaller eye. However, some individuals of *L. eques* cannot be

separated from some of *L. lepidion* by this maxillary–eye relation, the head or orbit size in relation to the standard length, or the meristic differences (apart from the possibility of differences in the number of lateral line scales, which I have not been able to investigate fully) (Table 3; Templeman, MS, 1970, tables 2–6).

Collett (1905), in a direct comparison between a specimen of *L. lepidion* from Nice in Christiania Museum and a specimen of *L. eques* of similar length from the Faroe Channel, showed that in the specimen of *L. lepidion* the eye was slightly smaller than in that of *L. eques* but that other characteristics appeared to be the same in both species. He concluded that *L. lepidion* from the Mediterranean was so similar to the North Atlantic *L. eques* that the two forms are probably identical.

Roule (1919), without documentation, uses the name *H. lepidion* var. *eques* Günther for a specimen from the Mediterranean off Monaco (evidently *L. lepidion* (Risso)) and for a specimen from the Bay of Biscay (evidently *L. eques* (Günther)) and says that this form must be considered as an abyssal variety of *H. lepidion* (Risso). Other specimens from the Mediterranean he names *H. lepidion*. In an appendix to Roule's paper, Vaillant gives seven Mediterranean specimens including Roule's Mediterranean specimen as *H. lepidion* (Risso). Evidently Roule but not Vaillant had difficulty in assigning all the Mediterranean specimens in his sets to *L. lepidion*, believing that one had the characteristics of *L. eques* but was not willing to give *L. eques* specific rank. Roule's Mediterranean specimen of *H. lepidion eques* was one of three *Lepidion* specimens taken at Station 392 on April 23–26, 1894. (In his text Roule gives August 23–26 in error.) Only two of these specimens are now available (Templeman, MS, 1970, *L. lepidion*, No. 9*j* and 9*k*, table 2, and No. 2 and 9, table 5). Although Roule's individual cannot be identified with certainty, it is probably No. 2(9*j*), which, apart from an orbit length at the upper end of the range for this species, is typically *L. lepidion* in its morphometric and meristic characteristics.

Norman (1936), in his review of *Lepidion* species, says that *L. eques* is very closely related to or perhaps identical with *L. lepidion*.

Grey (1956) includes both *L. lepidion* and *L. eques* as *L. lepidion* and says that, though the two forms may be distinct, they are closely related and cannot be separated by a study of the literature alone.

Raimbault (1963), without documentation but after examining specimens of *L. lepidion* that he caught in the western Mediterranean and comparing them with descriptions of *L. lepidion* and *L. eques* from the literature, found that some of his specimens belonged to *L. lepidion* for certain characters said to be of specific importance (in relation to *L. eques*) and to *L. eques* for others. He therefore did not regard the two species as distinct, and would include both in *L. lepidion*.

My investigations of these two species show many differences such as the lower means of meristic characters in *L. lepidion*, which may be related

to the warmer and possibly to the saltier environment of the Mediterranean as compared with the North Atlantic.

Differences may exist in numbers of scales along the lateral line. Very few *Lepidion* trawl specimens are captured with adequate scale covering for accurate scale counts along the lateral line and very few of these counts have been made. For *L. lepidion*, Vinciguerra (1883) says 155 scales along the lateral line and Günther (1887), 155–160. Günther only had one specimen and his 155 count presumably came from Vinciguerra's record.

For *L. eques*, Günther (1887) reports 180 rows along the lateral line, Koehler (1896) ca. 180, Holt and Calderwood (1895) 180 (but this is not original, all *L. eques* meristic counts of these authors being copied from Günther, 1887), and Koefoed (1927) ca. 180 for a specimen of 300 mm.

In view of these records and of the lower meristic counts generally for *L. lepidion*, the scale counts of the latter species are probably lower than for *L. eques*, but in view of the great variability of fish scale numbers under different temperature conditions a larger number of counts might show overlapping for this character.

The morphometric characters that differ most significantly are mostly directly or indirectly related to the length of the head or the size of the eye or both.

It is apparent to the eye and from the many points of resemblance in important characters, in which there are no absolute and obvious genetic differences but only those of degree, which could be phenotypic, that *L. eques* and *L. lepidion* are much more closely related to each other than to the other *Lepidion* species of the North Atlantic.

In view of the great resemblances and overlapping of the values for meristic and most morphometric characters, the few differences that occur between *L. eques* and *L. lepidion* would probably be considered as of subspecific rather than specific significance in commercial forms with widely overlapping distributions. Relative head size usually depends on growth rate, slow growing members of a species often but not always having larger body parts such as heads, eyes, and fins. Differences in body form may also be related to sexual maturity, depth of habitat, and other factors. Smaller heads and fins, usual in northern populations, are correlated with higher meristic counts in these populations (Martin, 1949; Barlow, 1961). The differences between the Mediterranean *L. lepidion* and the more northern and cooler water *L. eques* agree with this generalization except that usually relatively large heads are accompanied by relatively large eyes and the large headed *L. lepidion* have relatively small eyes.

The growth rates for these *Lepidion* populations are unknown and only for the northeast Atlantic population is even a small amount of knowledge available on size at sexual maturity, and this only for females. The areas in which *L. eques* and *L. lepidion* are found are, however, well separated and because the adults are normally found only in water deeper than the sill at the entrance to the Mediterranean there are unlikely to be adult migrations

between the two areas. The possibility, however, remains of some larval movement inward in the surface waters or some larval or small fish movement outward in the deeper water. This is unlikely to affect greatly the populations being compared here, but lepidions of the *L. eques* and *L. lepidion* type fish captured in the Atlantic closer to the entrance of the Mediterranean might have intermediate characters. The greater postorbital lengths in *L. lepidion* than in *L. eques* are not paralleled by a corresponding increase in the postorbital length of *L. eques* of the northwest Atlantic over those of the northeast Atlantic although, as in *L. lepidion* but to an intermediate degree, the horizontal diameter of the orbit is less in *L. eques* of the northwest than of the northeast Atlantic.

It is possible that if a larger number of *L. lepidion* and *L. eques* or specimens of this type caught in the Atlantic closer to the Mediterranean were examined, the postorbital lengths would overlap between these two species. It is possible also that with more complete information on the depth and other environmental relationships, growth, and sexual maturity of the species that an explanation other than that of species difference can be found for the difference in this character. Meanwhile it would be unwise to make *L. eques* a synonym of *L. lepidion*.

LEPIDION GUENTHERI, LEPIDION EQUES, AND *LEPIDION LEPIDION*

Meristic Characters

Considering only my counts (Table 3), *L. guentheri* is included within or overlaps with the range of *L. lepidion* for second dorsal rays, anal rays, and vertebral number. The means for *L. guentheri* tend to be higher ($P > 0.05$) for second dorsal rays and vertebral numbers and are higher ($P < 0.05$) for anal rays. The means of the second dorsal and of the anal fin-ray numbers of *L. guentheri* and *L. eques* differ ($P < 0.01$ and < 0.005). Also, they differ ($P < 0.001$) in vertebral number. For pectoral rays *L. guentheri* is within and below the lower part of the range of both *L. eques* and *L. lepidion*, the *L. guentheri* mean differing from that of *L. eques* ($P < 0.001$) and of *L. lepidion* ($P < 0.005$). The number of pelvic rays in *L. guentheri* is usually one below that of *L. lepidion* and *L. eques*, being seven and eight in the following numbers of individuals for these three species respectively: 7,1: 1,12: 0,39 (Templeman, MS, 1970, table 2). *Lepidion guentheri* possesses five long rays in the first dorsal much more often than *L. eques* and *L. lepidion*, in six out of eight individuals in my counts, whereas all 52 of my *L. eques* and *L. lepidion* had four long rays although Koefoed (1927) reports a five for a small *L. eques*. Günther (1887) also reports four(five) for *L. lepidion* but apparently examined only one specimen, which has a first dorsal count of 1+4 (Templeman, MS, 1970, table 2, item 9*a*). Günther's "(5)" is presumably copied from Vinciguerra (1883), who states that there are five rays in the first dorsal, the first of which is rudimentary (i.e., 1+4) (Templeman, MS, 1970, table 2, item 9*n*). Gill rakers are more numerous on the average in *L. guentheri*, the range for the

latter being included in the upper part of the range of *L. lepidion* and overlapping and extending beyond the upper part of the range of *L. eques*. The means for gill-raker numbers differ between *L. guentheri* and *L. eques* ($P < 0.001$) and between *L. guentheri* and *L. lepidion* ($P < 0.005$). The pyloric caeca are more numerous in *L. guentheri*, 15–17 in my counts of eight specimens and 15–17 for all authors compared with 8–13 for *L. eques* and 8–10 for *L. lepidion*. Including Maul's two counts of 17 with my counts for *L. guentheri*, the mean pyloric caecum count for this species (Table 3) differs greatly ($P < 0.001$; $t = 15.44$ and 16.85 for 46 and 17 degrees of freedom, respectively) from those of *L. eques* and *L. lepidion*.

Morphometric Characters

The orbit in *L. guentheri* is much smaller than in *L. lepidion* and *L. eques* — horizontal diameter range in my measurements in percentages for these three species respectively: 4.1–5.2, 6.5–7.9, and 7.3–8.9 SL; and 16.4–21.8, 27.6–32.1, and 32.6–38.5 head length (HL) (Fig. 10, 11; Templeman, MS, 1970, tables 3–6). For many characters, the larger size of *L. guentheri* in my measurements than of *L. eques* or *L. lepidion* and the occurrence of only one small *L. guentheri* in the lower part of the size range of the other two species offer difficulties of comparison in view of the evidence for allometry in *L. eques*. For the horizontal diameter of the orbit the regression line of negative allometry for *L. eques* would pass well above that for *L. guentheri* as also would the line for *L. lepidion* if it kept parallel to but below *L. eques* as indicated. Also, the horizontal diameter of the orbit of the smallest *L. guentheri*, which is approximately as small as the smallest *L. lepidion* and slightly smaller than the smallest *L. eques* examined, lies well below that for the latter two species. Because many of the larger females of *L. eques* in the northeast Atlantic are mature and because some of the smaller *L. eques* and *L. lepidion* have been caught at great depths, there is no reason at present to suppose that *L. eques* and *L. lepidion* of this area normally attain the same large sizes as *L. guentheri*.

The head length and the interorbital width in relation to the standard length in *L. guentheri* are relatively greater than in *L. eques*, although there is some overlapping, but are approximately in the same size ranges as those of *L. lepidion*. In *L. eques*, for head length the slight allometric increase with increasing fish length would, if projected, run below the *L. guentheri* range.

The snout length in *L. guentheri* is within and above the upper part of the range of this character for *L. eques* and *L. lepidion* both in relation to standard length and head length. The apparent positive allometry in *L. eques*, however, could reverse this situation if the *L. eques* line were extended to *L. guentheri* lengths, but there is no evidence for the existence of such large *L. eques*.

The postorbital length of the head of *L. guentheri* is above the range of this character in *L. lepidion* and even more for *L. eques*, especially in relation to the head length. For this character also there is no important allometric effect so that the differences are real in spite of the size differences.

The barbel length (Fig. 11–12) is greatest in *L. guentheri* and, considering the probable negative allometry of this character in relation to head length, the range of barbel length in relation to head length for *L. guentheri* is exceedingly well separated from those of *L. eques* and *L. lepidion*. The relative length of the pelvic fin in *L. guentheri* is distinctly greater than in *L. lepidion* and about twice as great as in *L. eques*. For relative length of pelvic fin in *L. eques* there is a definite decrease in the allometric regression line with increase in fish length so that at the projected position of this line at *L. guentheri* lengths the relative pelvic length in *L. guentheri* would be almost $2\frac{1}{2}$–3 times as great as in *L. eques* (Fig. 12).

The least height of the caudal peduncle is also distinctly greater in *L. guentheri* with the ranges well separated for the larger specimens (Fig. 12).

The greatest height of body (Fig. 12) increases rapidly with increase in standard length, but the line of rapidly increasing allometry of this characteristic of *L. eques* and *L. lepidion* in relation to the standard length lies above that of *L. guentheri*.

The distance from snout to midanus and that from the snout to the first ray of the first dorsal in *L. guentheri* (Fig. 13) are (except for the smallest specimen) both above the range of *L. eques* and overlap with and extend above the range of *L. lepidion*. The distance from the base of the pelvic to the anterior insertion of the anal is greatest in *L. guentheri* but overlaps with the upper part of the range of the other two species. It appears, however, that for all these three characters there is enough positive allometry with increasing fish length that at *L. guentheri* fish lengths the *L. eques* line would pass through or near the *L. guentheri* range.

There are also differences, sometimes very great, when morphometric characters are compared with other characters than the usual standard or head length. The interorbital width, snout length, and especially the postorbital length of *L. guentheri* in relation to the horizontal diameter of the orbit are each well above the range for *L. lepidion* and especially above that for *L. eques* (Fig. 14). The range of least height of the caudal peduncle expressed as a percentage of the distance between dorsal and caudal fins in *L. guentheri* includes only the highest value for *L. lepidion* and is considerably above all values for *L. eques* (Templeman, MS, 1970, tables 3–6). The pelvic length as a percentage of pectoral length is very greatly different in *L. guentheri* (156–189) than in *L. eques* and *L. lepidion* (65–96). In *L. eques* (there are only two records for *L. lepidion*) this relationship has strong negative allometry from ca. 94.5 at 140 mm SL to ca. 67 at 340 SL. Thus, because the *L. guentheri* standard lengths involved are all (except one) 413–554 mm, the actual differences are much greater than those recorded above, the values for *L. guentheri* at the largest sizes being 4 times the projected values of *L. eques* (Fig. 14).

The shortest distance (between transverse parallels) that the posterior base of the last anal ray is anterior to the posterior base of the last ray of the second dorsal (Fig. 14) and also the relative length of the longest gill raker (Templeman, MS, 1970, tables 3–6) are relatively less in *L. guentheri* (except

for the gill-raker length of the smallest) than in *L. eques* and *L. lepidion*, but it appears likely that this is the result of negative allometry.

In preserved *L. guentheri*, the lateral line is a darker brown than in the preserved *L. eques* and *L. lepidion* that I have seen.

In seven specimens of *L. eques* the sharply downward-turning portion of the lateral line begins 1.3–7.6 (average 4.0)% SL in front of the anterior insertion of the anal (Fig. 1, 2; Templeman, MS, 1970, tables 3, 4; distances were measured between parallel perpendicular planes). In 10 specimens of *L. lepidion* it begins 1.6–6.5% SL in front of and in one specimen 0.6% SL behind the anterior insertion of the anal (overall average 2.7% SL in front) (Fig. 3; Templeman, MS, 1970, table 5). In six specimens of *L. guentheri* it begins 1.2–3.7% SL behind and in one specimen 0.4% SL in front of the anterior insertion of the anal (overall average 1.7% SL behind) (Fig. 4, 5; Templeman, MS, 1970, table 6).

Posteriorly, the distance from the point where the sharply downward-turning portion of the lateral line changes to a shallower curve is 2.5–5.5 (average 4.3)% SL behind the anterior insertion of the anal in *L. eques*, 1.3–6.8 (average 4.2)% SL behind in *L. lepidion*, and 6.7–10.5(average 8.7)% SL behind in *L. guentheri* (Fig. 1–5; Templeman, MS, 1970, tables 3–6).

Similarly, the most anterior point at which the lateral line is transversely equidistant from dorsal and anal fin-ray bases is 6.8–9.5 (average 8.1)% SL behind the anterior insertion of the anal fin in *L. eques*, 6.1–10.5 (average 7.9)% SL in *L. lepidion*, and 14.3–18.6 (average 15.8)% SL in *L. guentheri*.

Discussion and Conclusions

Meristic characters

For my counts there are absolute differences between *L. guentheri* and *L. eques* and especially *L. lepidion* in numbers of pyloric caeca and, apart from one specimen of *L. guentheri* and one of *L. lepidion*, in pelvic fin-ray numbers, with little overlapping and highly significant or significant differences in mean numbers of pectoral fin rays and gill rakers. There are also real differences in mean numbers of first dorsal rays.

There are significant differences between *L. guentheri* and *L. lepidion* in mean numbers of anal fin rays and highly significant differences between *L. guentheri* and *L. eques* in mean numbers of second dorsal and anal fin rays and vertebrae.

For *L. guentheri*, Günther (1862, 1887) reports 210 rows of scales along the lateral line, Johnson (1862) ca. 200, and Maul (1952) ca. 205. For the specimen from Madeira, 536 mm SL (Templeman, MS, 1970, table 6, No. 7), I obtained a count of about 215 oblique rows of scales just below the lateral line. This count was probably correct within about ±5. This range of lateral line scale rows from ca. 200 to 215 is greater than the 180 reported for *L. eques* and the 155–160 for *L. lepidion* in the few specimens examined for this character.

It is apparent in the *L. eques–L. lepidion–L. guentheri* group that increases

in the size of the eye tend to reduce the actual postorbital length rather than the snout length. Thus the expansion for the larger eye is backward rather than forward.

In the truly meristic characters, second dorsal, anal, and vertebral numbers, *L. guentheri* is intermediate between *L. eques* and *L. lepidion* and usually closer to *L. eques* from west of Ireland than to the more northerly and westerly *L. eques* from colder water. Hence in these meristic characters, *L. guentheri* is related to the warmer water section of the *L. eques–L. lepidion* complex and the differences in these particular meristic characters in the three species could be of an environmental nature.

The higher first dorsal and the lower pelvic fin-ray numbers and higher pyloric caeca numbers of *L. guentheri* seem to be real differences, outside the range of the kind of variation noted for the truly meristic characters above. The higher lateral line scale counts of *L. guentheri* and the higher gill-raker numbers in *L. guentheri* may also be real differences. It may be argued that the very large sizes of *L. guentheri* in relation to those of *L. eques* and *L. lepidion* were responsible for the higher numbers of gill rakers and pyloric caeca in *L. guentheri*. However, the smallest *L. guentheri* comparable in length to the smallest *L. eques* and *L. lepidion* possessed 16 gill rakers (Templeman, MS, 1970, table 2), the same number as most of the large *L. guentheri* and considerably more than the averages for *L. eques* and *L. lepidion*; and it possessed about 17 pyloric caeca, the highest number found in *L. guentheri*. Also in *L. eques*, the only species for which fairly large numbers were available, there is no trend toward higher numbers of gill rakers and pyloric caeca with increase in fish length in the 129–336 mm SL length range examined.

MORPHOMETRIC CHARACTERS

The smaller orbit, the greater values for the postorbital length in relation to the diameter of the orbit, the much longer pelvic fin, both in relation to the standard length and to the length of the pectoral, and the greater height of the caudal peduncle of *L. guentheri* provide distinct separations in these characters between *L. guentheri* and the *L. eques–L. lepidion* group.

The caudal fin is rounded in *L. eques* and *L. lepidion* as shown in figures of *L. eques* in Günther (1887), Holt and Calderwood (1895), and Schnakenbeck (1933; copied from Holt and Calderwood, 1895). For *L. lepidion* there are figures showing a rounded caudal fin in Risso (1810), in Vinciguerra (1883), in Goode and Bean (1896; copied from Vinciguerra, 1883), in Torchio (1961), and (since in most specimens of these fishes the posterior part of the caudal fin is injured) most convincingly in a life photograph from more than a mile below the sea surface in Edgerton (1955).

Text statements that the caudal fin is rounded also appear for *L. eques* in Günther (1887), Holt and Calderwood (1895), Koehler (1896), and Goode and Bean (1896), and for *L. lepidion* in Risso (1810) and Goode and Bean (1896). Strangely, Vinciguerra (1883), for *L. lepidion*, although showing a figure with a rounded caudal fin, says that the tail fin is truncated. This appears

to have been copied in error from Günther's (1862) description of *H. lepidion* = *L. guentheri* (Giglioli). In many of the St. John's specimens, also, although often the posterior fringe of the caudal fin is imperfect, it is evident that the caudal fin is rounded.

In contrast to the rounded caudal fin of *L. eques* and *L. lepidion*, in *L. guentheri* the caudal fin is truncate. Günther (1862) for his *H. lepidion* (= *L. guentheri*) says that the caudal fin is truncated (also Johnson, 1862). Similarly Maul (1952) in figure and text indicates a distinctly truncated caudal fin. Also all seven large specimens of this species with relatively perfect caudal fins seen by the author (Fig. 4, 5; Templeman, MS, 1970, table 6) had truncated caudal fins but with the greatest length dorsally.

The evidence presented earlier demonstrates that the beginning and end of the sharply downward-turning portion of the lateral line from a dorsal to a near median position and the point where the lateral line reaches the median position are more posterior in *L. guentheri* than in *L. eques* and *L. lepidion*.

The new evidence, therefore, reaffirms and extends the previous evidence of Giglioli (1880), Günther (1887), and Maul (1952) for a specific difference between *L. guentheri* and the *L. lepidion–L. eques* group.

LEPIDION SP. AND OTHER LEPIDIONS

Meristic Characters

Forster's two very large specimens of *Lepidion* from the mouth of the Bay of Biscay, which he attributed, without meristic or morphometric documentation, to *L. guentheri* (Forster, 1968), will be shown not to be *L. guentheri*, and, to distinguish them for comparison with other North Atlantic species, tentatively will be called *Lepidion* sp. or *L. ?schmidti*. These specimens are widely different from all other North Atlantic lepidions in second dorsal fin-ray number, 47–48 as against 55–60 (my counts only) for *L. eques*, 54–59 for *L. lepidion*, and 54–58 for *L. guentheri* (Table 3). They are even more distinct in anal fin-ray number, 40–41 compared with 50–54 for *L. eques*, 48–51 for *L. lepidion*, and 49–53 for *L. guentheri*. Dr D. M. Cohen (personal communication, November–December 1967) has provided (by direct observation, not radiographs) a second dorsal count of 51 and an anal count of 47 for a 471 mm SL *L. guentheri* from Madeira, which is now in the museum at Monaco. I have not been able to study this specimen. This narrows the gap considerably between *Lepidion* sp. and *L. guentheri* for the second dorsal, but not much for the anal. It will be necessary to study radiographs of this specimen to confirm the anal number, which on external counting is sometimes one too low, and to decide whether or not the low counts are due to vertebral or other abnormalities. There are usually no difficulties in counting the second dorsal of large specimens by direct observation. The same reasoning, also, presumably applies to Maul's (1952) counts of 51–56 second dorsal and 46–50 anal rays in five specimens of *L. guentheri* from Madeira. In No. 3520 for example (Tem-

pleman, MS, 1970, table 2, No. 10*h*), which was included in Maul's range of 46–50 for the anal, I obtained a count of 52 rays for the anal on the radiograph although I had previously counted only 50 on the fish.

The first dorsal in *Lepidion* sp., 1+5 in both specimens, is one greater than is customary in *L. eques* and *L. lepidion* and closer to *L. guentheri*, where 1+5 is the usual number but does not occur in all specimens. In vertebral number, at 54–56 the two *Lepidion* sp. are a little less than *L. eques* (57–62), *L. lepidion* (56–58), and *L. guentheri* (57–59). In pelvic fin rays, seven, they are the same as is usual in *L. guentheri* and one below *L. eques* and usually *L. lepidion*. In gill-raker numbers, at 12–14 they tend to be lower than *L. eques* (13–16), and *L. lepidion* (13–18), and are lower than *L. guentheri* (16–18). In pyloric caeca,16, the one specimen counted is similar to *L. guentheri* (15–17) but higher than *L. eques* (8–13) and *L. lepidion* (8–10).

In my counts of scale rows above the lateral line, I found 26–27 in *Lepidion* sp. compared with a number usually within the range of 14–18 noted for the *L. eques*, *L. lepidion*, and *L. guentheri* examined. Because of the great difference in size of *Lepidion* sp. and the other species, however, and the presence of small scales immediately in front of the first dorsal there is some possibility of error through not counting in exactly the same way in the various species. Hence further careful checking needs to be done to substantiate this difference.

Although the meristic counts of *Lepidion* sp. differ from those of other North Atlantic lepidions they are similar for the dorsal, anal, and pelvic fins and vertebrae to those of *L. schmidti* Svetovidov, 1936 (Templeman, MS, 1970, table 2) from Japan, and also close enough in pectoral fin-ray and gill-raker numbers to be the same species.

Morphometric Characters

For many morphometric characters (Fig. 10–14; Templeman, MS, 1970, table 6), *Lepidion* sp. often includes *L. schmidti* within its range and, for the other characters available, its range lies so close to the values for *L. schmidti* that it is possible to believe that they are the same species. The relatively greatest body height is less in *L. schmidti*, but *L. schmidti* is considerably smaller and this character increases with standard length. The head length and the least height of the caudal peduncle are greater in *L. schmidti*, but the difference from *Lepidion* sp. is less than that occurring within *L. eques* for these characters. Also, there is no apparent change in the relative lengths of these characters with increase in fish length in *Lepidion* (Fig. 10, 12). The distance from the pelvic fin base to the anal fin is considerably less in *L. schmidti*, but allowing for the strong positive allometry (Fig. 13) there is no real difference between *Lepidion* sp. and *L. schmidti* in this character.

For morphometric characters the range of *Lepidion* sp. and the available values for *L. schmidti* are usually within, overlapping, or close to the range of *L. guentheri*. Especially to be noted are the similarity with *L. eques* and *L. lepidion* in possessing a small orbit diameter with related relatively high snout and postorbital lengths, and the high values for the least height of the caudal

peduncle and for the snout length in relation to the orbit diameter. *Lepidion* sp. and available *L. schmidti* values are different from those for *L. guentheri* in the lower values, comparable with those for *L. lepidion* for length of the pelvic fin, and considerably lower than those for *L. guentheri* but considerably greater than those for *L. eques* and *L. lepidion* for the pelvic length expressed as a percentage of the pectoral length (Fig. 12, 14). The length of the pelvic fin and the pelvic fin length as a percentage of pectoral length appear to be strongly negatively allometric in *L. eques*, but there is no evidence of this tendency in *L. guentheri* in sufficient degree to destroy the differences between this species and *Lepidion* sp. for these characters.

Discussion and Conclusions

Forster's *Lepidion* sp. is separable from the other North Atlantic species of *Lepidion* especially by the considerable differences in the counts of anal fin rays, by the differences in ranges of the counts for the second dorsal fin, and possibly by the higher number of scale rows above the lateral line, but these and other available meristic counts are similar to those of *L. schmidti* from Japan. My counts of about 26–27 scale rows above the lateral line for *Lepidion* sp. from Plymouth were independently checked by Mr Alwyne Wheeler of the British Museum in the manner used by me, i.e., according to Hubbs and Lagler (1958). Mr Wheeler reported (personal communication, November 1, 1968): "I have examined Forster's giants again and counted the scale rows as described by you. I count 25 and 26 on the larger and 27 and 25 on the smaller (left and right sides respectively). You will, however, note that this was to the mid-line in front of the dorsal and included a number of small scales. These small scales are present along the dorsal fin bases, but if one counts to the dorsal rays, omitting the small scales, one gets a count of 15, 16 or 17. These are large, regularly arranged scales only."

Dr A. N. Svetovidov (personal communication) says that on the holotype of *L. schmidti* Svetovidov he has recently counted approximately 26–28 scales above the lateral line, not including the scale of the latter, upward and forward to the midline in front of the first dorsal fin. Hence *Lepidion* sp. and *L. schmidti* are similar in this respect also.

For *Lepidion* sp. from Plymouth the lateral line scale count (actually the number of oblique rows counted immediately below the lateral line) was about 212–214 for the larger specimen and about 216–217 for the smaller. These figures represent the final two counts in each case. No great accuracy can be claimed for these counts but the numbers are evidently approximately in the *L. guentheri* (200–215) rather than in the *L. eques* (180) or the *L. lepidion* (155–160) range. This overlapping with *L. guentheri* is surprising in view of the considerably larger scale count for *Lepidion* sp. than for *L. guentheri* above the lateral line.

The number of pyloric caeca of *L. schmidti* Svetovidov is not available. Dr Svetovidov (personal communication) says that he has recently examined

the holotype of *L. schmidti* and no stomach, intestine, or intestinal caeca remain.

The other meristic characters of *Lepidion* sp. and most of the morphometric characters either overlap with or, with allowance for allometry, could be those of *L. guentheri* rather than of *L. eques* or *L. lepidion*. *Lepidion* sp. has, however, considerably lower values for pelvic fin length than *L. guentheri*.

In Forster's *Lepidion* sp. (my observations and Fig. 6) the caudal fin is truncate, as it is in *L. schmidti* (Svetovidov, 1936, fig. 1) and in *L. guentheri* as discussed previously but not in *L. eques* and *L. lepidion*.

In Forster's *Lepidion* sp. the colour is brownish grey with the edges of the fins darker. The lateral line is dark brown, almost black in one specimen and closer in depth of colour to *L. guentheri* than to *L. eques*, or *L. lepidion*.

Although it is easy to think that Forster's giant lepidions should be adult *L. guentheri*, it must be concluded on the basis of present evidence that they are not, because, although in most characteristics they resemble this species, there are some absolute differences. For the present they are more appropriately placed as *L. ?schmidti*. However, such small numbers are involved that there are doubts which need to be removed by the study of more specimens of *L. guentheri*, *L. ?schmidti*, and *L. schmidti* Svetovidov. The two giant *L. ?schmidti* at 1150 and 1230 mm total length (TL) are mature females. The largest *L. guentheri* available for examination, females 584 and 605 mm TL, showed no indications of sexual maturity. Considerably larger specimens of *L. guentheri*, 734 and 810 mm SL, were taken at Madeira and recorded at the Funchal Museum (Templeman, MS, 1970, table 1), and one of 889 mm TL was recorded by Johnson (1862). Thus, there is no reason at present to believe that *L. guentheri* could not grow as large as *L. ?schmidti*. If *L. ?schmidti* is a true species of the Atlantic, one wonders why smaller specimens have not been taken. The fate of Johnson's largest specimen of *L. guentheri* is unknown, and Mr G. E. Maul (personal communication, November 2, 1968) says that the two large specimens recorded cannot be found at the museum at Funchal.

The study of larger specimens would be useful in helping to decide whether the length of the pelvic fin in *L. guentheri* declines with increasing fish length as it does in *L. eques* but not in *L. guentheri* in the specimens available. If it declined sufficiently in *L. guentheri* with increase in fish length the difference between *L. guentheri* and *L. ?schmidti* in this respect would be reduced or eliminated.

The range of meristic numbers in the two specimens of *L. ?schmidti* is small and data from new individuals of this form would very likely narrow the gap in meristic characters between the ranges of *L. guentheri* and *L. ?schmidti* and enable a new assessment to be made of the closeness of their relationship. New information, which removed differences between *L. guentheri* and *L. ?schmidti*, would also remove differences between *L. guentheri* and *L. schmidti* Svetovidov.

Svetovidov's (1936) drawing of *L. schmidti* shows an anal fin without the concave outline typical of other *Lepidion* species, but Svetovidov (personal communication, 1968) says that the middle rays of the anal fin of *L. schmidti* are shorter than the anterior and especially the posterior rays.

Characters described for *L. schmidti* agree in meristic values and are close enough to *Lepidion* sp. in morphometric values (apart from some doubts about relative head length and least height of the caudal peduncle) for *Lepidion* sp. to be *L. schmidti* and tentatively they may be called *L. schmidti* pending further study. Preferably this would be done by an investigator studying the genus on a worldwide scale since possibly other specimens of *L. schmidti*, which are unrecognized or unreported at present, are present in some of the world's museums.

DISTRIBUTIONS AND DOUBTFUL NOMINAL RECORDS OF *LEPIDION* SPP.

Lepidion eques (Günther, 1887)

Records of *L. eques* (Fig. 15; Templeman, MS, 1970, table 1) show that it is distributed from the Faroes area and west of Scotland and southwest of Ireland to the Bay of Biscay, westward from the Faroes along the Iceland–

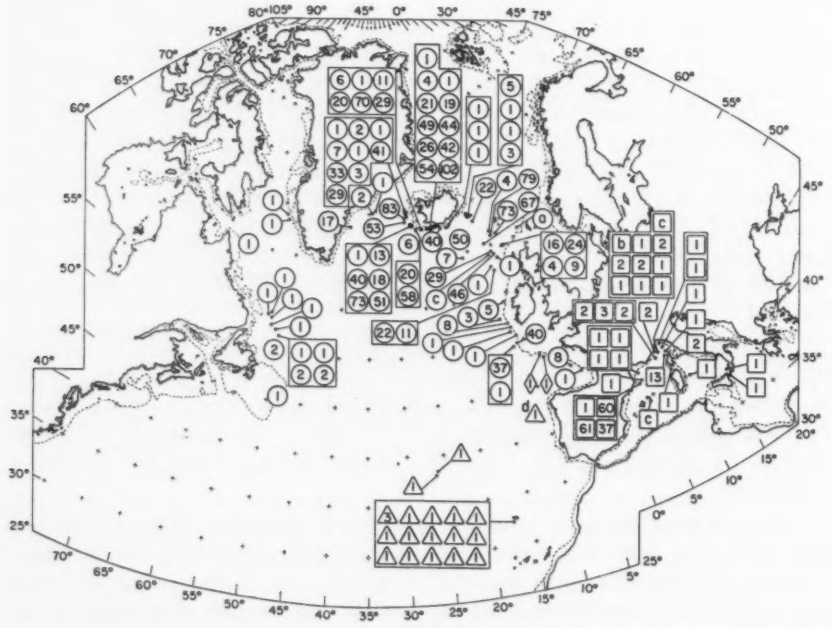

Fig. 15. Distribution of captures in the North Atlantic of the various *Lepidion* species. ○, *Lepidion eques*; □ *L. lepidion*; △, *L. guentheri*; ◇, *L. ?schmidti*; *a*, numerous; *b*, very rare; *c*, number doubtful; *d*, exact position unknown. (The two specimens of Table 1, No. 14, were captured too late to be included in this figure.)

Faroe Ridge, south and southwest of Iceland to east and west Greenland and, with the 17 St. John's records (Fig. 15, Table 1), from northern Labrador along the continental slope to the southeastern Grand Bank.

In the northeast and north-central Atlantic and in west Greenland (Templeman, MS, 1970, table 1), these fish were taken at considerable depths, all but 1 of 37 catches of moderately large to large numbers (11–102 individuals) and including fish over 200 mm in length being from 500 to 1300 m. Only one of these very successful sets, off the east coast of Greenland, was in shallower water (330 m). Captures have been recorded down to 1850 m and small specimens in shallower water (127 m). In the Canadian area the new records range only from 385 to 775 m but few trawl sets have extended deeper than 730 m.

Total lengths of the specimens from the Canadian area ranged from 155 to 332 mm and those from the more eastern Atlantic distribution from ca. 55 to 440 mm.

Bottom temperatures in the areas of capture of 11 or more specimens in one set in the northeastern Atlantic and south and southwest of Iceland ranged between 5.2 and 9.4 C, whereas in the Canadian area they were several degrees lower, 3.4–4.4 C. These, however, are the highest deepwater temperatures available in the western Atlantic areas in which *L. eques* has been found to occur.

On four occasions in the Canadian area, two individuals were taken in one set (Table 1). The others are individual records. In the northeast Atlantic (Templeman, MS, 1970, table 1) greater numbers, up to 102, were taken in one set, and off east Greenland 17 in one set. The two west Greenland records are of single individuals.

Like the other records of this species by most authors, Roule's (1919) record of an individual of *H. lepidion* var. *eques* Günther from the Mediterranean (Templeman, MS, 1970, table 1) is not documented as to body proportions and meristic characters. From the same western Mediterranean station and set for which Roule (1919) in his text and table reported a specimen of *H. lepidion* var. *eques* but no other *Lepidion* specimens, Vaillant in an appendix to Roule's paper reported three *H. lepidion* (Risso) but no *H. lepidion* var. *eques*. I have accepted Vaillant's museum examination and record in preference to Roule's apparently more casual or shipboard record and consequently do not include this Mediterranean specimen of Roule's as evidence of a Mediterranean distribution for *L. eques* but rather have included it with *L. lepidion*.

Richard (1934) and Guiart (1935), without description or further documentation, reported a large *Lepidion* specimen from the Azores, called *Lota lepidion* by the former and *H. lepidion* var. *eques* by the latter author. The specimen weighed 3 kg and was eaten. Two specimens of *L. eques* from the Newfoundland area, 290 and 309 mm TL, weighed 154 and 180 g from 50% alcohol. Two specimens of *L. guentheri* from Madeira, 478 and 584 mm TL, weighed 780 and 1700 g out of 10% formalin. The longest *L. eques* recorded was 440 mm in length (Templeman, MS, 1970, table 1). The weight of the above specimen from the Azores, therefore, was about 5 times as great as

the longest *L. eques* known but comes within the size range of *L. guentheri*, for which specimens are recorded up to 810 mm SL and 889 mm TL. From its size and location the specimen was probably *L. guentheri* and is included with *L. guentheri* in Templeman (MS, 1970, table 1). It could also have been similar to *L. ?schmidti*.

LEPIDION LEPIDION (RISSO, 1810)

Lepidion lepidion has been reported from the northern part of the western Mediterranean (Fig. 15; Templeman, MS, 1970, table 1). Although most authors have reported it rare, deepwater sets (Raimbault, 1963; Maurin, 1968) have shown it to be moderately plentiful in some areas. It has been reported from 600 to 2230 m and is most abundant below 750 m. Small specimens of *L. lepidion* <100 mm long have been reported at shallower depths, 150–175 m. Maximum sizes may be smaller than in *L. eques* because the greatest length reported for *L. lepidion* is 340 mm, whereas for *L. eques* lengths up to 440 mm are reported (Templeman, MS, 1970, table 1). However, much larger numbers of *L. eques* have been caught and measured.

In addition to the lepidions recorded in Templeman (MS, 1970, table 1), Edgerton (1955) shows the photograph of a *Lepidion* presumably *L. lepidion* (Risso) taken from a bathyscaphe off Toulon in the northwestern Mediterranean at a depth of more than 1600 m. It has a rounded caudal fin of the *L. eques–L. lepidion* type and, in this area, is presumably *L. lepidion*.

LEPIDION GUENTHERI (GIGLIOLI, 1880)

Lepidion guentheri has been recorded mainly from Madeira, and also from the west coast of Spain and Portugal and the Azores (Fig. 15; Templeman, MS, 1970, table 1).

It has also been reported without documentation of meristic or morphometric characters from west of Ireland and north and northwest of the Hebrides (Blacker, 1962, 1968), from northwest of the Hebrides by Postel and Du Buit (1965), and from the mouth of the Bay of Biscay (Forster, 1968).

I have examined Blacker's (1962) specimens now at the British Museum (Templeman, MS, 1970, tables 1, 4) and they are not *L. guentheri* but typical *L. eques*. It is assumed that Blacker's (1968) 10 specimens of *L. guentheri* from north and northwest of the Hebrides, an area from which only *L. eques* has previously been reported, are also *L. eques* and not *L. guentheri*. Mr A. C. Wheeler of the British Museum assures me that he has examined them and that they are *L. eques*.

Four specimens from northwest of the Hebrides were examined by Postel and Du Buit (1965) and named *H. güntheri*. No actual data are provided by which the accuracy of this identification can be judged. However, the figure of *L. guentheri* drawn from these specimens shows that these fish are not *L. guentheri* but *L. eques*. The large eye in relation to head length, the shortness of the pelvic in relation to the pectoral, the shape of the lateral line, with the

beginning of the steep downward slope well anterior to the origin of the anal, the smallness of the ratio of caudal peduncle height to eye length, and the whole appearance of the fish, leave no doubt that these specimens are typical *L. eques* and not *L. guentheri.*

I have also examined at the British Museum (Table 2, 4) Forster's (1968) two large specimens, which he named *L. guentheri,* but as demonstrated previously in this paper they are not *L. guentheri* nor any North Atlantic *Lepidion* previously known or described, but tentatively *L. schmidti.*

Lepidion guentheri has also been reported, but vaguely and apparently incorrectly, from the Mediterranean. Günther (1862) gives the distribution of *H. lepidion* as "Mediterranean, Madeira." Günther's actual specimen (later named *H. guentheri* by Giglioli) was from Madeira, but he believed it to be the same species as *L. lepidion* (Risso) from the Mediterranean. Hence apparently the Madeira location was for his specimen whereas the Mediterranean location was for Risso's and similar specimens of *L. lepidion.*

Canestrini (1872) in his Pesci d'Italia lists *H. lepidion* (Risso) from the Mediterranean but attributes to it exactly the same meristic characters given by Günther (1862) for *H. lepidion* from Madeira (= *L. guentheri*). There is no evidence that this is a Mediterranean record. Canestrini states that Risso found this species at Nice (but Risso's species is *L. lepidion*), and Canestrini like Günther (1862) was evidently equating Günther's *H. lepidion* from Madeira (actually *L. guentheri*) with Risso's *L. lepidion* from the Mediterranean.

Giglioli (1880) named Günther's actual specimen of *H. lepidion,* above, from Madeira as *H. guentheri* and said that Mediterranean specimens (Risso's *G. lepidion*) were very different.

Vinciguerra (1883), after describing *H. lepidion* (Risso) from the Mediterranean, in comparison with *H. guentheri* Giglioli, says "Dr Günther, to whom I reported the evidence of the table representing *H. lepidion,* wrote me that having examined specimens from the Mediterranean, perfectly identical to those from Madeira, the existence is not improbable of two species of the genus *Haloporphyrus* in the aforesaid area."

Again Günther (1887) for *L. guentheri* says that this species has been obtained off the coasts of Madeira and Portugal and also in the Mediterranean.

Carus (1893) in Prodromus Faunae Mediterraneae, quoting Günther, lists the Mediterranean as part of the habitat of *L. guentheri.*

Norman (1936) for *L. guentheri* says "Habitat — Mediterranean and adjacent part of the Atlantic."

In spite of all the above I can find no real evidence of a record of *L. guentheri* from the Mediterranean. Dr D. M. Cohen (personal communication, December 14, 1967) says that he did not find any Mediterranean material of *L. guentheri* in the museums in London, Paris, Monaco, Florence, and Genoa. Prof E. Tortonese, Director of the Museu Storia Naturale Genova (letter, July 9, 1968) says that "as far as I know — after a perusal of the literature and study of material — *L. lepidion* is the only Mediterranean species."

Günther (1862) was in error as he thought that his *H. lepidion* from Madeira (Giglioli's *H. guentheri*) was the same as Risso's *G. lepidion* from the Mediterranean. Apparently Günther (1887) was still in error for the distribution of *L. guentheri*, and followed his old (1862) distribution list for *H. lepidion* (Giglioli's *H. guentheri*).

Canestrini (1872), Carus (1893), and Norman (1936) evidently merely copied Günther, in error, for the Mediterranean distribution of *L. guentheri*. Vinciguerra's record of a letter from Günther saying that he had examined specimens of *H. guentheri* from the Mediterranean is more difficult to understand. Vinciguerra published his remarks in 1883, and Giglioli in 1880 had assigned Günther's *H. lepidion* to *H. guentheri*. The *L. lepidion* (Risso) described by Vinciguerra (1883) was captured in 1879. It therefore appears probable that Günther wrote Vinciguerra before he was aware or before he agreed with Giglioli that his Madeira *Lepidion* was a new species, distinct from Risso's *L. lepidion*.

Lepidion schmidti Svetovidov, 1936

The fish that I have tentatively assigned to *L. schmidti* (=*L. guentheri* of Forster, 1968) were found in the North Atlantic at the mouth of the Bay of Biscay at 2002 and 2058 m. At 1150 and 1230 mm TL they are by far the largest North Atlantic lepidions known, the next largest two, which are *L. guentheri* from Madeira, being 810 mm SL and 889 mm TL (Templeman, MS, 1970, table 1). The holotype of *L. schmidti*, however, 497 mm TL (Svetovidov, 1936), was taken in Sagami Bay, Japan, at a depth of 550–700 m. I have noted no other records of this species in the literature and Dr Svetovidov knows of no others.

It is not unusual for a deepwater species to occur over a wide expanse of the oceans. For example, the related morid species *Antimora rostrata* occurs in deep water in the North and South Atlantic and in northern and southern parts of the Pacific Ocean (Grey, 1956). As another example, the deepwater skate *Raja richardsoni*, first collected in New Zealand (Garrick, 1961), has been reported also from the northeast Atlantic (Forster, 1965, 1968).

Other Nominal Records of *Lepidion*

Lepidion lepidion

Canestrini's (1863) record of *Lota lepidion* (Risso) from the Mediterranean, still taken as a record of *Lepidion lepidion* (Risso) by some authors, was named in error and was shown to refer to *Molva molva* by Günther (1863), Giglioli (1882), and Vinciguerra (1883).

Pérès (1958) reported a *Haloporphyrus* ca. 25 cm long as seen near the bottom during a bathyscaphe dive at 1340–1350 m in the canyon of Cape Sicié near Toulon. Presumably this specimen was *Lepidion lepidion*.

There are four nominal records of *Lepidion lepidion* from the northwest Atlantic: two of 25 and 45 mm in length, from the stomach of an *Alepisaurus*

ferox, April 29, 1963, 39°20′N, 44°50′W; and two, each of 42 mm in length, from the stomach of an *Alepisaurus brevirostris*, June 7, 1963, 39°23′N, 68°33′W (Haedrich, 1964). I examined these four specimens, now in the Museum of Comparative Zoology at Harvard, and could not find any close resemblance between them and *Lepidion*. In these fish (Fig. 16) the number of pelvic rays (ca. 10–11) is considerably greater than in *Lepidion* (7–8). There are six to seven rays in the first dorsal. The anal and second dorsal rays are approximately equal in number (second dorsal rays ca. 61–62 and anal ca. 61 in the one specimen examined). The rays (especially of the anal) are more numerous than in *Lepidion* and the anal rays begin farther forward in relation to the beginning of the second dorsal. The base of the pectoral is well in front of the first ray of the first dorsal. The pelvic fin has many long rays instead of two as in *Lepidion*. The ventral procurrent rays of the caudal extend forward slightly beyond the transverse line from the posterior ray of the second dorsal so that no transverse section of the caudal peduncle is free of rays dorsally and ventrally. The lower jaw extends in front of the upper and the mouth is at an angle of about 45° with the horizontal. The eye is almost round rather than obviously oval. The first long ray of the first dorsal is not extended to a great length. All of these and other characteristics are different from those of *Lepidion*. Haedrich's fish are consequently not *Lepidion* but are similar to *Gargilius vitellius* Koefoed (Koefoed, 1953).

LEPIDION SP.

Maul (1952) described and figured a fish of which he obtained two specimens, one 43 and the other 48 mm SL, from the stomach of *A. ferox*, and doubtfully assigned them to *Lepidion* sp. Through the kindness of Mr Maul, I had the opportunity of seeing these specimens but, because of their very poor condition, did not think it possible to improve on Maul's detailed description. There are, however, a number of characters considerably different from *Lepidion*. The eye is rounded rather than oval as in *Lepidion*. There is no long ray of the first dorsal (although it is not certain that at this small size it would be present). The number of rays in the anal fin (58) is only one less than that in the second dorsal. In my counts (Table 3) the highest number of rays in the anal fin of any North Atlantic *Lepidion* was 54, 6 below the highest second dorsal number of 60 and 4 below Maul's *Lepidion* sp. In *Lepidion* specimens (Templeman, MS, 1970, table 2) the second dorsal exceeds the anal count by 2–8, and usually by 4–7. The number of scales between the lateral line and the first dorsal (10) is considerably lower than in any North Atlantic species of *Lepidion*. The lower jaw is at least as long as the upper, not considerably shorter as in *Lepidion*. Maul says they apparently possess no pyloric caeca. This would be another difference from *Lepidion*. I conclude that these fish are too different from *Lepidion* to belong to this genus.

CONFUSION BETWEEN YOUNG *LEPIDION EQUES* AND *GARGILIUS*

Saemundsson (1926) under *H. eques* Günther says that it was first thought that this was a new species of the cod family and it was given the name *Gargilius*

(after a famous Roman gourmand). It is evident, however, from the description of *G. vitellius* in Koefoed (1953) and from my remarks above on Haedrich's specimens, that *Gargilius* is very different from *Lepidion*. Also possibly in the same category and referring to *Gargilius* rather than *Lepidion* is Saemundsson (1927) when he says referring to *H. eques* "some pelagic fry have also been taken on [sic = over] great depths off the south coast of Iceland (Schmidt)." *Gargilius* was caught in *Thor* Station 180 at 61°34′N, 19°05′W in 1904 (Koefoed, 1953). This may have been the capture noted as I have been unable to find a record of the pelagic fry of *Lepidion* referred to above.

Another record, because of its small size and the errors that have been made in naming fish of this size as *Lepidion*, needs documenting before it can be fully accepted as *L. eques* rather than *Gargilius* or some similar species. This is the 50–60 mm *H. eques* (Saemundsson, 1939, 1949) that was reported to Saemundsson by Dr A. F. Brunn as caught by the *Dana* on July 6, 1927, off the south coast of Iceland (Templeman, MS, 1970, table 1). I have, however, been unable to trace this specimen. Mr J. Nielsen writes that it is not in the collections of the Universitetets Zoologiske Museum at Copenhagen.

PHOTOGRAPHIC RECORDS OF UNIDENTIFIED DEEP-SEA MORIDAE OF THE WESTERN ATLANTIC

Brundage et al. (1967) show a photograph of a morid or a deep-sea cod taken in 1963–64 near bottom at about 2500 m approximately at 41°45′N, 65°00′W east of the mouth of the Fundian Channel of the Gulf of Maine. These Moridae were dark fish ca. 26–45 cm in length and were moderately abundant. Marshall and Bourne (1967) show a photograph of a similar morid fish apparently taken in about 2600 m on the continental slope south of Nova Scotia.

I compared the dorsal outline of these fish with those of *L. eques* and of the blue hake, *A. rostrata*, and the fish are evidently not *Lepidion* but *A. rostrata*. St. John's staff have caught these blue hake, 40–56 cm long, in fair numbers on longline at about 2200 m near the southern area mentioned above and also large blue hake at similar depths on the continental slope of the Nova Scotian Shelf. The blue hake is not blue but black when caught at these depths.

SEXUAL MATURITY

As noted previously in this paper for *L. eques*, body form (especially the greatest height of body in females) may change with the attainment of sexual maturity.

Through the kindness of Dr G. Krefft and his assistant Miss C. Lübben, German data on the sexual maturity of *L. eques* from the southern Icelandic slope were made available (Table 5). These records were taken in April when some spawning was proceeding. Some of the fish, both males and females of 28–29 cm and longer, were close to spawning or had spawned and some females of 22–23 cm, most of 24–25 cm, and all of 26–27 cm and over were beginning to mature.

FIG. 16. One of four specimens from the northwest Atlantic, nominally *Lepidion lepidion* (Haedrich, 1964) but here identified as *Gargilius vitellius* or one of its relatives.

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TABLE 5. Length-frequency distributions of males and females according to stage of maturity for 210 specimens of *Lepidion eques* taken from the southern Icelandic slope, April 27–29, 1965 (positions between 63°00′–63°28′N and 22°06′–26°16′W, bottom depths 550–800 m, bottom temperatures ca. 5.2–6.5 C).[a]

Total length (*cm*)	No. males				No. females								Total male	Total female	Total male + female
	I	II	III	IV	I	II	III	IV	V	VI	VII	VII–II			
11	1	–	–	–	–	–	–	–	–	–	–	–	1	–	1
12–13	1	–	–	–	1	–	–	–	–	–	–	–	1	1	2
14–15	1	–	–	–	3	–	–	–	–	–	–	–	1	3	4
16–17	3	–	–	–	3	–	–	–	–	–	–	–	3	3	6
18–19	6	–	–	–	4	–	–	–	–	–	–	–	6	4	10
20–21	9	–	–	–	9	–	–	–	–	–	–	–	9	9	18
22–23	11	–	–	–	6	4	–	–	–	–	–	–	11	10	21
24–25	5	1	–	–	6	9	–	–	–	–	–	–	6	15	21
26–27	12	2	–	–	–	13	–	–	–	–	–	–	14	13	27
28–29	6	4	1	–	–	14	1	–	–	–	–	–	11	15	26
30–31	8	4	2	–	–	9	4	–	–	–	1	–	14	14	28
32–33	8	2	2	–	–	2	4	–	1	–	0	1	12	8	20
34–35	–	5	1	–	–	–	5	–	1	1	1	–	6	8	14
36–37	–	2	2	–	–	–	–	–	1	–	1	–	4	2	6
38–39	–	–	–	2	–	–	–	–	–	–	–	–	2	–	2
40–41	–	–	–	3	–	–	–	–	–	–	–	–	3	–	3
42	–	–	–	1	–	–	–	–	–	–	–	–	1	–	1
Total	71	20	8	6	32	51	14	–	3	1	3	1	105	105	210

[a]These data were forwarded to me by Dr G. Krefft, Bundesforschungsanstalt für Fischerei, Institut für Seefischerei, Hamburg, and were taken by his assistant Miss C. Lübben. Total lengths were measured to the centimetre below and hence are on the average a half centimetre too low. I, immature; II, beginning to mature (some of the larger specimens may be recovering from spawning (VII)); III, ripening; IV, advanced ripening; V, almost ready to spawn; VI, spawning; VII, spent; VII–II, recovering from spawning.

TABLE 6. Gonad development and maturities at various lengths of *Lepidion* specimens examined by this author. *Lepidion eques* from the Faroe Channel (F), west of Ireland (I), west of Iceland (Ic), and Newfoundland area (N); *L. lepidion* from the Mediterranean (L); *L. guentheri* from Madeira and west coast of Spain and Portugal (G); and *L. ?schmidti* from Plymouth Laboratory (S). When available, the number of the month of capture is placed immediately after the area.

Total length (*cm*)[a]	Male, greatest width 1 testis (*mm*)	Female immature or immature condition[b]		Female maturing or mature	
		Ovary volume (*cc*)	Egg diameter (*mm*)	Ovary volume (*cc*)	Egg diameter (*mm*)
		L. eques			
14–15	(F8; 0.2, 0.5, 0.5), (I4; 0.5)	(F8; 0.1), (I4; 0.03)	(F8; 0.05), (I4; <0.05)	–	–
16–17	(I4; 1.2), (F8; 1.1)	(F8; 0.15)	(F8; <0.1)	–	–
18–19	(N9; 1.1)	–	–	–	–
20–21	–	(N5; 0.01)	(N5; <0.1)	–	–
22–23	(N5; 1.0)	(Ic7; 0.2)	(Ic7; 0.1)	(I4; 1.0)	(I4; 0.6)
		(N9; <0.05), (N11; 0.1)	(N9; <0.1), (N11; <0.1)		
24–25	(F8; 1.1, 1.3), (I4; 2.7)	(N10; 0.15)	(N10; <0.1)	(I4; ca. 4, ca. 7)	(I4; 0.6, 0.9)
26–27	(F8; 0.9, 1.1)	(N10; 0.3)	(N10; 0.1)	–	–
28–29	(F8; 1.7), (N10; 2.4, 2.7)	–	–	(I2; ca. 6)	(I2; 0.6)
30–31	(N8; 2.3, 2.4)	–	–	(I2; ca. 12, ca. 15)	(I2; 0.6, 0.6)
32–33	(N4; 2.0)	(N9; 2.2)	(N9; 0.1)	–	–
34–35	–	–	–	–	–
36–37	(I2; 4.0)	–	–	–	–
		L. lepidion, L. guentheri,* and *L. ?schmidti			
17–18	–	(L4; 0.2)	(L4; 0.1)	–	–
24–25	(L; 1–9)	(L; 0.8)	(L; 0.1)	–	–
26–27	(L; 1.0)	(L3; 0.8), (L4; 0.8), (L9; 0.6)	(L3; 0.1), (L4; 0.1), (L9; 0.1)	–	–
28–29	(L; 1.0)	–	–	–	–
45–47	–	(G8; ca. 3)	(G8; 0.2)	–	–
48–52	(G9; 6.5)[c]	(G2; ca. 3), (G; ca. 8)	(G2; ca. 0.2), (G; ca. 0.1)	–	–
53–54	(G8; 3)[d]	–	–	–	–
58–61	–	(G7; ca. 6), (G; ca. 10)	(G7; ca. 0.1), (G; ca. 0.1)	–	–
115–123	–	–	–	(S6; large)	(S6; 0.25)
				(S6; large)	(S6; 0.25)

[a]Natural measurements with a ½-cm offset, the first space being 1½ cm.

[b]Immature condition designates a stage of sexual maturity for young fish that have spawned, but in which, before ripening again, the gonad passes through a recovering condition resembling the immature stage.

[c]Volume 2 testes ca. 1 cc and fish was presumably mature or maturing.

[d]Testes brownish grey and thin. Fish appeared to be immature or immature condition.

In the *L. eques* from the northwest and northeast Atlantic examined by me (Table 6) all females from west of Ireland from 22–23 cm and longer taken in February and April were ripening but none were spent.

None of the five females from the Newfoundland area of 22–23 to 32–33 cm in length showed any indication of ripening or maturity. Bottom temperatures at which the *L. eques* from the southern Icelandic slope were taken were ca. 5.2–6.5 C, and Holt and Byrne's bottom temperatures from which some of the west of Ireland fish came were 10.1 C. The bottom temperatures for the *Lepidion* sets of the northwest Atlantic were 3.4–4.4 C. In the North Atlantic, therefore, for these *L. eques* high temperature is associated with sexual maturity at a smaller length.

The northwest Atlantic females of 22–23 cm and over were caught in September, October, and November, and the period of capture may have been responsible for some of the lack of development but not all, because the largest egg diameters for the *L. eques* of the Newfoundland area in October–November were 0.1 mm (egg volume 0.0005 mm^3) whereas on February 3 the west of Ireland fish had egg diameters of 0.6 mm (egg volume 0.11 mm^3). There is no evidence, therefore, at present of sexual maturity and spawning of *L. eques* in the Newfoundland area. The ovary of the largest female was slightly enlarged but the eggs were little developed.

The four females of *L. lepidion*, 25–26 cm long (Table 6), showed no evidence of sexual maturity. The months of capture of three of these were March, April, and September but that of the other is unknown. If the spawning season and length at sexual maturity in the Mediterranean are close to that for the west of Ireland *L. eques*, these *L. lepidion* specimens should have been ripening in March or April. Hence they were presumably immature.

The five female *L. guentheri*, 45–61 cm long (Table 6), were in an immature condition with regard to egg size, and their small, thin-walled ovaries in relation to the size of the fish gave no indication of sexual maturity.

The two large *L. ?schmidti* from the Plymouth Laboratory were sexually mature, possessing large ovaries with a tough and apparently contracted wall as though spent. The eggs at 0.25 mm diam were enlarging toward another spawning.

For the males examined, stage of maturity could not be determined by a casual examination of the testes.

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Distribution of *Anotopterus pharao* in the North Atlantic and Comparison of Specimens of *A. pharao* from the Western North Atlantic with Those from Other Areas

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TEMPLEMAN, W. 1970. Distribution of *Anotopterus pharao* in the North Atlantic and comparison of specimens of *A. pharao* from the western North Atlantic with those from other areas. J. Fish. Res. Bd. Canada 27: 499–512.

Two records of *Anotopterus pharao* Zugmayer from the Labrador Shelf, and three from the Grand Bank and vicinity, are the only records of captures from these areas and the only ones from the Newfoundland area apart from four Soviet records from Flemish Cap. One of the Grand Bank captures, October 1952, and a Labrador capture, September 1954, are the earliest records from the Newfoundland area.

Meristic and morphometric data indicate that these Newfoundland area specimens and two other west Greenland and one southeast Greenland specimens of *Anotopterus* examined by me are referable to *Anotopterus pharao* Zugmayer, as are all known specimens from the eastern Atlantic, and are similarly specifically related to Pacific and antarctic specimens. However, a few differences will need to be resolved by studying a larger number and size range of specimens from all areas of distribution.

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INTRODUCTION

TWO SPECIMENS OF *Anotopterus pharao* Zugmayer, 1911, taken off Labrador and one taken north of the northeastern Grand Bank by research vessels of the Fisheries Research Board of Canada St. John's, Nfld., Biological Station, and two taken by Newfoundland trawlers on the eastern Grand Bank, are the only records known to me from the Newfoundland area apart from four Soviet records from Flemish Cap.

The Grand Bank specimen of October 1952 and the Labrador specimen of September 1954 are the earliest records from the Newfoundland area (Appendix 1).

The history of this species, including the various scientific names that have been assigned to it, is given by Hubbs et al. (1953), who compared the meristic and morphometric characteristics of Atlantic, Pacific, and antarctic specimens of *Anotopterus* and concluded that they all belonged to the same species, *A. pharao* Zugmayer, which was distributed in an antitropical (bipolar) manner. Agreeing with Nybelin (1946) and Maul (1946), Hubbs et al. (1953) concluded that the family Anotopteridae with its single species, *A. pharao*, belongs to the order Iniomi, with close relationship with the Alepisauridae and the Paralepidae, probably closest to the Alepisauridae. Marshall (1955) and Rofen (1966) agreed with these conclusions.

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Information on distribution is provided for all captures of *A. pharao* known to me from the North Atlantic (Fig. 1; Appendices 1, 2).

The only *Anotopterus* data from the North Atlantic available to Hubbs et al. (1953) and Marshall (1955) were from the southern part of eastern North Atlantic, especially Madeira. This was also true of Rofen (1966) apart from one medium sized specimen from the northwest Atlantic off New Jersey. Only a few morphometric data are available for this specimen for comparison with the specimens of Hubbs et al. (1953).

Thurow (1961) and Postolaky (1962) each provided some meristic and morphometric information on a specimen from west Greenland but there are as yet no such published data for specimens from the Newfoundland–Labrador area and information for the Greenland specimens is incomplete. In order to provide the necessary data for specific identity and comparison, therefore, for the northwest Atlantic and neighbouring areas, I have provided meristic and morphometric information on three *Anotopterus* from the Newfoundland area, two from west Greenland, and one from southeast Greenland (Table 1, 2). Some comparisons have also been made between these northwest Atlantic *Anotopterus* and specimens of *A. pharao* from the northeast Atlantic, South Atlantic, North Pacific, and Antarctic.

MATERIALS AND METHODS

The Newfoundland specimens were measured from 10% formalin after soaking in water for a few hours and the Greenland specimens from the British Museum were measured out of 70% ethyl alcohol. These fish are not perfect for length measurements as the muscles are stronger than the vertebral column and the preserved fish is likely to have many bends, which must be straightened carefully.

Unless stated otherwise, the methods of measurement and meristic counting are those of Hubbs and Lagler (1958). Apart from total lengths and standard lengths, which are the shortest distances between perpendicular parallel planes, other measurements are direct distances between points.

DISTRIBUTION IN THE NORTH ATLANTIC

In the northwest Atlantic, the presently known range of *A. pharao* lies between 40°00′ and 64°25′N and in the northeast Atlantic between 29°23′ and 45°20′–46°40′N (Fig. 1; Appendix 1, 2).

Depths of capture extend from near the surface to 2000 m or more for the fish and from 40–100 to 2250 m for occurrences in fish stomachs. Captures are recorded from 1.5–5 to at least 17 C but most of these records are from occurrences in fish stomachs.

Captures are recorded from west Greenland between April–May and early November, from the Newfoundland area between July and October, from the Bay of Biscay to west of the Strait of Gibraltar region between July and September, and from Madeira between September and April.

The 19 specimens from the Newfoundland and Greenland areas are mainly large, all except two, 427–about 825 mm standard length (SL), and the 33 northeast Atlantic specimens all small, 47.5–342 mm SL, except one, 759 mm SL (Appendix 1, 2).

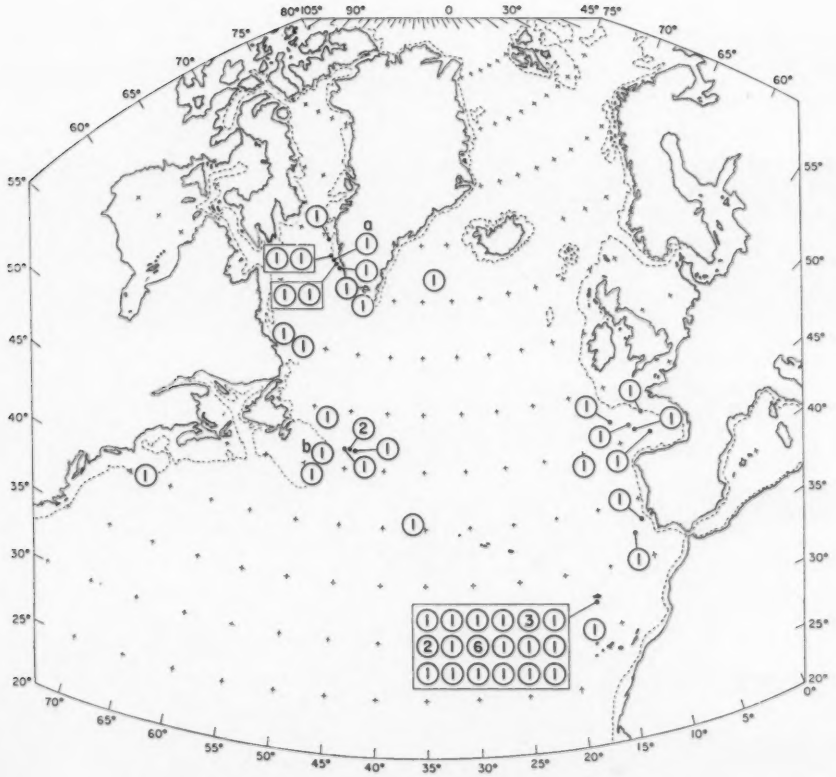

FIG. 1. Distribution of reported captures of *Anotopterus pharao* in the North Atlantic. (Numbers refer to the number of specimens captured — see Appendices 1 and 2; *a*, west Greenland, exact locality unknown; *b*, east Grand Bank, exact locality unknown. The map used for this figure was adapted from an outline chart of the North Atlantic Ocean on an azimuthal equal area projection centered at 40°N, 35°W, prepared at the Woods Hole Oceanographical Institution by M. J. Pollack and G. G. Pasley, April 1951. The 100-fath (183-m) isobath is shown.)

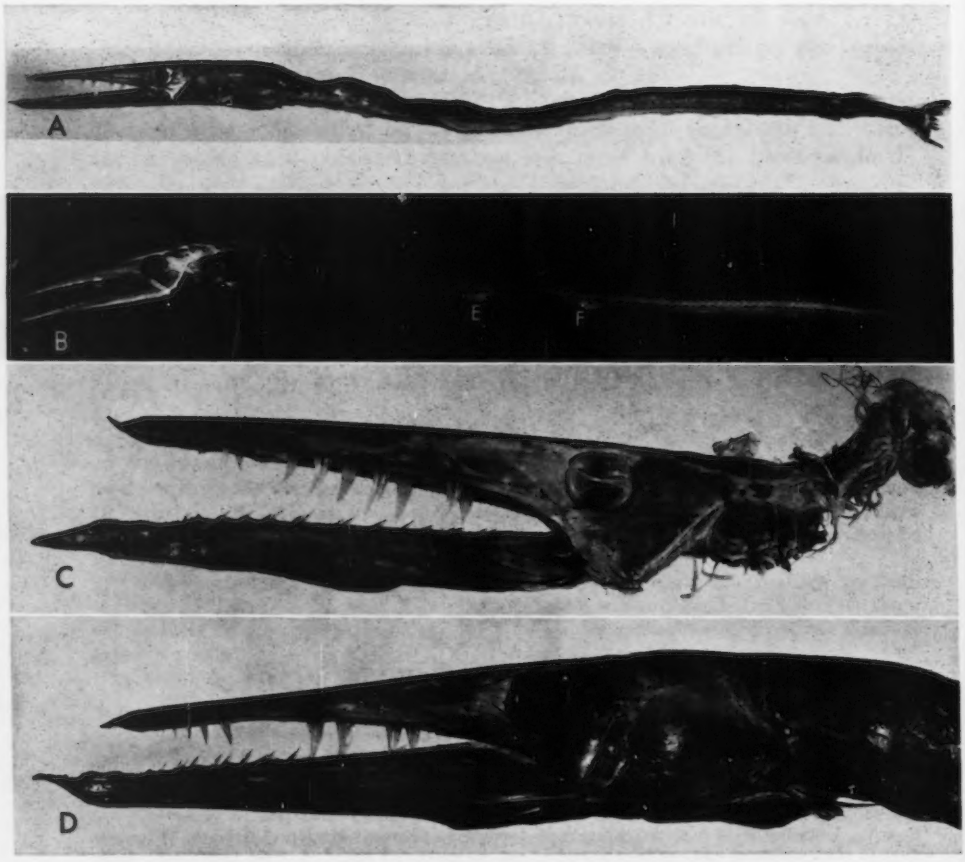

FIG. 2. Lateral views of *Anotopterus pharao*: A, 567 mm SL from east of southeast Greenland, Appendix 2, No. 1; B, radiograph of 278 mm SL specimen from Madeira, Table 1, No. 22; C, head only, from Hamilton Inlet Bank, Appendix 1, No. 8; D, head of specimen from Dana Bank, Appendix 1, No. 3. (Note the two small fish, E and F, in the stomach of *Anotopterus* B.)

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FIG. 3. Radiographs of lateral views of anterior portion of *Anotopterus pharao*: above, 734 mm SL from Dana Bank, Appendix 1, No. 3; below, 278 mm SL from Madeira, Table 1, No. 22. A, 2 sockets of missing sixth and seventh large palatine teeth. These were counted for this specimen. B, first vertebra. (Note that the first vertebra is shorter anteriorly than posteriorly. This is usual in *Anotopterus*.) C, anterior portion of a capelin in the stomach of the *Anotopterus*. There were nine capelin in the stomach of this specimen when captured.

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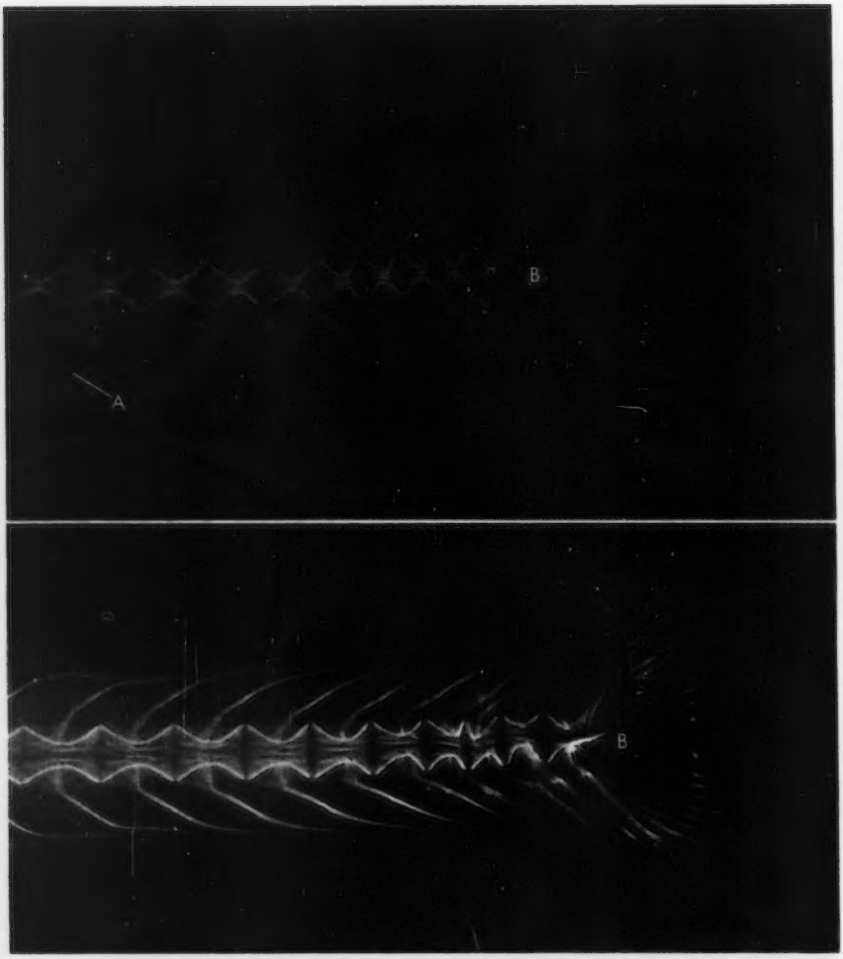

FIG. 4. Radiographs of posterolateral views of *Anotopterus pharao*: above, 688 mm SL from the Grand Bank, Appendix 1, No. 11; below, 278 mm SL from Madeira, Table 1, No. 22. A, the two most posterior anal rays, very close together and counted as one; B, the hypurals, which, together with the endostylar half-vertebra, clearly seen in the Madeiran specimen, were counted as the last vertebra.

MERISTIC CHARACTERS

Examination of the worldwide meristic data (Table 1), and elimination of the presumed errors (see discussion) indicates that the examination of larger numbers of specimens might show some differences in averages between areas. The number of vertebrae may be higher on the average in the antarctic specimens and the number of pelvic rays may be on the average lower in South Atlantic and antarctic specimens. The number of pelvic rays and anal rays may be highest in North Pacific specimens. The number of principal caudal rays (branched plus one unbranched above and below) is typically 19 (10 above and 9 below) and the number of branchiostegal rays typically 7. The fixed functional palatine teeth are highly variable in number and may average higher for South Atlantic and antarctic specimens but some variation in methods of counting between authors is to be expected (Fig. 2, 3).

MORPHOMETRIC CHARACTERISTICS

For most morphometric characteristics the ranges for *Anotopterus* specimens from the Newfoundland and Greenland areas include the values for Zugmayer's type specimen (Table 2, No. 10; Hubbs et al., 1953). The exceptions are the larger values in Zugmayer's type for the orbit (eye in Zugmayer's specimen), interorbital width, distance from snout to pelvic, and distance from pectoral base to pelvic base, and the smaller distances from pelvic base to anal base and pelvic base to midbase caudal (Table 2).

For almost all morphometric characteristics the Newfoundland and Greenland specimens are within or overlap the ranges of those from other areas, especially those of similar lengths (Table 2). In fish of approximately the same length, the postorbital length (6.6–7.5 SL) is lower than in specimens from the North Pacific (8.1–9.2 SL), the least height of the caudal peduncle (1.4–about 2.1 SL) is higher than in specimens from the antarctic (1.0–1.2 SL), and the greatest width of the body (about 1.8–about 2.8 SL) is lower than in specimens from the North Pacific (3.4–4.1 SL). The distances from snout tip to anterior insertion of the pelvic and from snout tip to anus are a little less (55.4–58.9 and 58.6–61.2 SL) in *Anotopterus* from the Newfoundland and Greenland areas than in smaller specimens from Madeira (60.3–62.3 and 62.4–63.5 SL). In the large North Pacific specimens the range for these measurements includes the western Atlantic range and overlaps with the Madeira range. Correspondingly the posterior distances from the pelvic base to the anterior origin of the anal base and from the pelvic base to midbase of the caudal are greater (29.8–33.8 and 41.1–43.7 SL) in the Newfoundland and Greenland specimens than in the smaller *Anotopterus* from Madeira (26.9–29.7 and 38.5–39.7 SL). These distances, however, are fairly similar in the western Atlantic and the large North Pacific specimens.

OTHER DATA

Dermal Caudal Keels

Only the three Greenland specimens, 567–734 mm SL, examined by this author had the skin suitably preserved, but there was no evidence of the dermal caudal keels (Rofen, 1966), characteristic of large specimens of *A. pharao*.

*TABLE 1. Meristic characters of *Anotopterus pharao* from the

Item no.	Authority	Year of capture	Locality	Position	
				Lat	Long
Western North Atlantic					
1	Nybelin (1946)	1935	Off W Greenland	64°25′00″N	53°30′00″W
2	Templeman	1964	Off Frederikshaab or on Dana Bank	–	–
3	Templeman	1964	Dana Bank	–	–
4	Postolaky (1962)	1959	W Greenland	ca. 62 29 N	ca. 51 19 30 W
5	Postolaky (1962)	1959	"	ca. 62 29 N	ca. 51 19 30 W
6	Thurow (1961)	1960	"	60 25 N	48 10 W
7	Templeman	1960	Labrador Shelf	55 27 N	56 36 W
8	Templeman	1954	Hamilton Inlet Bank	54 50 N	53 29 W
9	Postolaky (1962)	1960	Flemish Cap	46 31 N	44 51 W
10	Templeman	1952	E Grand Bank	44 00 N	49 05 W
11	Templeman	1953–58	"	–	–
12	Rofen (1966)	1958	E of New Jersey	40 00 N	68 14 W
Central and eastern North Atlantic					
13	Templeman	1964	E of SE Greenland	62 N	33 W
14	Collett (1896)	1888	Off Cape Finisterre	44 01 26 N	15 31 W
15	Nybelin (1948)	–	Off Portugal	38 25 N	10 23 W
16	Zugmayer (1911a, b)	1910	W of Strait of Gibraltar	36 54 30 N	11 49 W
17	Roule (1927)	1926	Madeira	–	–
18	Maul (1946)	ca. 1943–46	"	–	–
19	Maul (1952)	1946	"	–	–
20	Hubbs et al. (1953)	ca. 1943–46	"	–	–
21	Hubbs et al. (1953)	ca. 1943–46	"	–	–
22	Marshall (1955)	1944	"	–	–
23	Dr G. Krefft (personal communication, Jan. 7, 1969)	1968	NW of Canary Islands	29 23 N	18 10 W
Eastern North Pacific					
24	Hubbs et al. (1953)	1948	Off B.C.	Near 52 N	–
25	Rofen (1966)	1955	–	40 54 N	164 54 W
26	"	"	–	40 54 N	164 54 W
27	"	"	–	38 32 N	147 16 W
28	Hubbs et al. (1953)	–	Off Calif.	35 35 N	122 25 W
29	Rofen (1966)	1957	Off Calif.	35 N	124 W
30	Hubbs et al. (1953)	1951	–	31 45.4 N	152 12.6 W
Western North Pacific					
31	Hubbs et al. (1953)	1935–37	Kamchatka	–	–
32	Welander et al. (1957)	1953	Near Kiska Is.	51 20 N	176 36 E
33	Welander et al. (1957)	1953	Near Kiska Is.	51 20 N	176 36 E
34	Abe (1952)	–	Kamchatka or Hokkaido	–	–
35	Kobayashi and Ueno (1956)	1954	–	49 45 N	170 25 E
36	Hubbs et al. (1953)	1951	Hokkaido	42 55 N	145 42 E
South Atlantic and Antarctic					
37	Marshall (1955)	–	S Atl.	23 16 S	01 51.7 W
38	Dr G. Krefft (personal communication, Jan. 7, 1969)	1968	E of S America	34 01 S	47 39 W
39	"	"	"	34 01 S	47 39 W
40	"	"	"	36 37 S	51 32 W
41	Nybelin (1946)	1930	Weddell Sea	ca. 62 S	ca. 38 W
42	Marshall (1955)	1949–50	Ross Sea	69 S	170 E
43	Regan (1913); Marshall (1955)	1904	Off Coats Land	71 22 S	16 34 W

*Footnotes to this table are on p. 504.

western North Atlantic compared with those from other areas.

SL (*mm*)	No. fin rays: Pectoral	Pelvic	Anal[a]	No. caudal rays: Principal: Above	Below	Procurrent: Above	Below	No. vertebrae[b]	No. branchiostegal rays	No. functional fixed palatine teeth
ca. 700[c]	–	–	–	–	–	–	–	–	–	ca. 10
658	d	d	14	d	d	17[e]	16[e]	78	d	10
734	14	9	14	d	d	d	d	78	7	11
750[f]	13	9	14	–	–	–	–	78[b]	6–7	9[g]
870[f]	–	–	–	–	–	–	–	78[b]	–	13
840[f]	13	–	14	–	–	–	–	82[b,h]	–	8
810	14	11	15[i]	10	9	15	13	79	7	10
ca. 825[j]	–	–	–	–	–	–	–	–	–	11
ca. 700[c]	–	–	–	–	–	–	–	–	–	13
427	15	10	d	10	9	ca. 12	ca. 12	80	7	5
688	13	10	15	10	9	17	17	78	7	8
453	12–13	9	14	–	–	–	–	79	–	–
567	14	11[k]	13	d	d	d	d	80	d	7
253[f]	ca. 12–13	–	–	–	–	–	–	–	–	ca. 7
14[l]	–	–	–	–	–	–	–	–	–	4
154[m]	14[n]	4	12	2/16/6		–	–	–	6	–
271[m]	12	4	13	–	–	–	–	81[o]	–	6
265–342	10	9	15 or 16	19		18	18	–	7	6
47.5[l]	–	–	–	–	–	–	–	–	–	14
247	14	10	14	10	9	16–17	15–16	78[p]	7	8
260	14	10	14	10	9	16–17	15–16	79[p]	7	9
278	12	9	15	–	–	–	–	80[b]	–	6
87.4	–	–	–	–	–	–	–	81[b]	–	–
87[q]	–	–	–	–	–	–	–	–	–	5
332	15	10	14	–	–	–	–	80	–	–
510	13	10–11	14	–	–	–	–	80	–	–
276	13	11	16	–	–	–	–	80	–	–
299	ca. 14	ca. 10–11	16	10	9	–	–	–	7	4
238	14	11–10	14	–	–	–	–	79[r](76)[r]	–	–
113	14	11	15	10	9	15	14	–	7	8
861	12	10	15	10	9	14	14 or 15	79+ 1 or 2 centra	7	10
759	–	–	–	–	–	–	–	–	–	9
867	–	–	–	–	–	–	–	–	–	10
855	ca. 15	11	14	–	–	–	–	–	–	10
817	13	10–9	17	18		13	13	–	7	6
640	14	11	16	10	9	14	14 or 15	79[s]	7	10
100	14	9	15	–	–	–	–	–	–	13
190[t]	–	–	–	–	–	–	–	81[b]	–	–
195[t]	–	–	–	–	–	–	–	83[b]	–	–
160[u]	–	–	–	–	–	–	–	82[b]	–	–
650[l]	12	9	11	–	–	–	–	83[b]	–	14
732	14	9	14	–	–	–	–	83[b]	–	11
150[q]	–	–	–	–	–	–	–	–	–	13

Sex and Maturity

There is little in the literature on sex or maturity. Rofen (1966) says that the largest subadults examined, to 510 mm, were all males. Postolaky's (1962) two large specimens from southwest Greenland were both males. All four *Anotopterus* examined by me from Greenland and Labrador (Table 2), and in suitable condition for examination of the gonads, were females. These females had very small eggs, 0.1–0.2 mm diam, and the ovaries were narrow, flat, and thin-walled giving no indication that they had reached sexual maturity. The three smaller specimens of 567–734 mm SL had ovaries 2.5–4 mm wide and the largest specimen, 810 mm SL, an ovary 8 mm wide and 270 mm long.

Mr G. E. Maul, however, wrote me (December 16, 1968) that he had a ripe female *Anotopterus*, 759 mm SL, obtained near Madeira on September 19, 1961 (Appendix 2, No. 27).

Food

Blacker's specimen from Dana Bank, September 1964, 734 mm SL, contained, when I examined it at the British Museum, nine small adult capelin, *Mallotus villosus*, about 110–140 mm total length (TL) (Fig. 3C).

Footnotes to Table 1 on Pages 502–503

[a]In Templeman's counts, the last two anal rays very close together and counted as one.

[b]In Templeman's and other vertebral counts, including urostylar half-vertebra + hypural as one vertebra. Note: I have checked from radiographs the vertebral numbers of 80 and 83 in Marshall's (1955) specimens, No. 22 and 42.

[c]Total length estimated from head only.

[d]Unsuitable for this character.

[e]Assuming 10 principal caudal rays above and 9 below.

[f]Total length.

[g]Dr A. I. Postolaky (personal communication, Jan. 20, 1969) says, regarding the palatine tooth number of 11–12 reported in his paper for this specimen, that the number of fixed teeth extant is 9 and teeth lost according to vacant spaces were 2–3.

[h]Thurow (1961) gives 83 vertebrae for this specimen but he has kindly sent me the radiograph which I have checked and obtained a count of 82 vertebrae including the hypural.

[i]Last three rays very close together and counted as one.

[j]Standard length calculated from head only.

[k]9 in right pelvic.

[l]Total length without caudal.

[m]Estimated correct SL from Hubbs et al. (1953).

[n]14 in Zugmayer (1911a), 12 in Zugmayer (1911b).

[o]From Roule (1935).

[p]From Rofen (1966), whose two larger Madeiran specimens obtained from G. E. Maul had the same meristic counts as those of Hubbs et al. (1953) (although one differed slightly in SL) and were the same specimens.

[q]Length of head.

[r]For seven fish Rofen (1966) gives eight vertebral counts. These are the seventh and eighth counts and the seventh fish.

[s]From Abe (1952).

[t]Caught by *Walther Herwig*, pelagic trawl Feb. 14, depth ca. 2000 m, temperature 3.56 C, salinity 34.812‰, two specimens in one set, collector G. Krefft.

[u]Caught by *Walther Herwig*, pelagic trawl Feb. 16, depth ca. 2000 m, temperature 3.55 C, salinity 34.758‰, collector G. Krefft.

DISCUSSION AND CONCLUSIONS

DISTRIBUTION

The new records from the western and eastern North Atlantic presented in this paper are within the area of distribution north of the equator postulated for this species by Hubbs et al. (1953). In agreement with the hypothesis of Hubbs et al., also, most of the northern specimens are large and most of the southern specimens small. However, if small fish occur toward the south it is to be expected that the larger fish return south to spawn and their relative failure to be captured toward the south may be related to the lack of suitable facilities for capture or to lack of observation by scientists in the southern areas. *Alepisaurus ferox* is capable of taking much larger prey than the *Anotopterus* of 220–342 mm SL (one of 47.5) reported by Maul (Appendix 2). Haedrich (1964) reports *Alepisaurus brevirostris* of 439 and 585 mm SL from the stomach of this fish. Most of the food fish of *A. ferox* reported by Haedrich, however, were less than 160 mm, but this may be due to the lack of large food fish in the oceanic area fished. The lack of large *Anotopterus*, therefore, in the *A. ferox* at Madeira may mean that larger specimens of *Anotopterus* are not available or are very scarce in this area during the winter–spring period. However, the report by Mr G. E. Maul of a ripe female *A. pharao*, 759 mm SL, taken near Madeira in September (see previous text) shows that spawning of *Anotopterus* does occur in this area in autumn. *Alepisaurus ferox* appears to take its food mainly from the upper layers and the larger *Anotopterus* may be located more deeply where it is not often sampled by efficient gear. Mr Maul has informed me that he intends to publish an account of this fish.

In view of the extension of warmer Atlantic water so much farther northward in the eastern than in the western North Atlantic it is surprising that *A. pharao* has been reported almost 18° of latitude farther north in the northwest than in the northeast Atlantic. It is to be expected that captures will eventually be reported farther north in the northeast Atlantic.

Of the 19 specimens of *Anotopterus* from the western Atlantic (Appendix 1) 5 from fish stomachs were heads only, 1 was the posterior part of the body only, and 5 were entire; 8 were from bottom trawls. It is remarkable that often only the heads are reported. It may be suspected that the head region is selected for attack, but the discovery of a tail portion from a deepwater chimaera (Appendix 1, No. 12) leads me to suspect that tail portions may be present in stomach contents but are more likely than heads to be passed over without recognition of species.

Since it is often obtained from fish stomachs and less often from bottom trawls, *Anotopterus* is presumably pelagic as its form would indicate. It is consequently usually taken by fish such as *Alepisaurus* living in the surface layers or by those such as cod, which make considerable vertical migrations. Even those caught in bottom trawls may have been taken while the trawl was being pulled upward or have been regurgitated from fish stomachs during the ascent of the trawl.

TABLE 2. Morphometric characteristics of *Anotopterus pharao* from Newfoundland, Greenland, and other areas. (Except where indicated otherwise all body parts are expressed in percentages of the standard length. Specimen numbers are from Appendices 1 and 2. In data from literature all data calculated from figures were omitted.)

Item no.	Body part	Greenland (British Museum, Templeman) W (4)	Greenland (British Museum, Templeman) W (5)	Greenland (British Museum, Templeman) SE (1)	Newfoundland (Templeman) Labrador (10)	Newfoundland (Templeman) Grand Bank (16)	Newfoundland (Templeman) Grand Bank (17)	Off New Jersey (Rofen, 1966) (18)	W of Strait of Gibraltar (Hubbs et al., 1953[a]) (10)	Madeira (Hubbs et al., 1953[b]; Marshall, 1955; Rofen, 1966) (14, 26, 27)	N Pacific (Hubbs et al., 1953; Rofen, 1966)	N Pacific (Hubbs et al., 1953; Kobayashi & Ueno, 1956; Welander et al., 1957; Rofen, 1966)	Antarctic (Hubbs et al., 1953[a]; Marshall, 1955)
1	Greatest total length (*mm*)	694+	777+	603+	[c]	456+	743+	–	165	–	–	–	–
2	SL: snout to end hypural (*mm*)	658	734	567	810	427	688	453	154	244–278	238–332	510–867	632–732
3	Head: length[d]	21.0	23.5	23.8	20.5	22.9	21.2	24.4	21.3	20.0–25.3	23.6–26.8	22.5–24.3	20.6–23.0
4	Orbit: horizontal diameter	2.2	1.9	2.2	1.8	2.2	1.9	2.4[e]	2.9[e]	2.2–3.0	2.7[f]	1.9–2.2	1.9
5	Interorbital width[g]	1.5	1.5	1.5	1.3	1.5	1.3	1.4	2.3	1.3–1.8	1.4–1.9	1.3–1.5	1.2–1.3
6	Orbit to mandibular angle	2.2	2.8	2.4	2.3	2.4	2.1	–	–	2.5	2.4	2.0–2.7	–
7	Orbit to nostril	1.2	1.5	1.2	1.1	1.2	1.0	–	–	1.3–1.4	1.5	1.0–1.5	–
8	Snout: length	12.4	14.1	14.1	11.8	13.6	12.6	14.7	13.0	12.2–15.9	14.3–15.9	12.4–14.1	12.0–12.5
9	Upper jaw: snout to angle of gape	12.4	13.9	14.1	13.2	[c]	[c]	–	–	16.5–17.1	14.0	13.8–16.1	–
10	Bony mandible: length	15.4	17.3	17.3	[c]	16.8	15.4	–	–	18.3–18.4	17.2	15.5–16.1	–
11	Mandibular process: length	[c]	1.7	1.1	[c]	1.8	1.6	–	1.9	1.5–2.2	1.2	1.0–1.5	1.9
12	Postorbital: length	6.6	7.5	7.4	6.9	7.3	7.0	7.5	–	6.8–7.4	7.1–8.3	8.1–9.2	–
13	Body: greatest height	[c]	5.9	5.5	ca. 6.5	[c]	[c]	5.6	5.8	5.2–5.4	4.5–6.6	5.8–7.3	3.3–5.1
14	Caudal peduncle: least height	1.4	1.6	1.4	ca. 1.7	ca. 2.1	ca. 1.9	1.5	1.8	1.5–1.8	1.6–1.8	1.6–2.1	1.0–1.2
15	Head: greatest width	2.4	2.5	2.7	2.6	ca. 3.3	ca. 2.4	–	–	2.9–3.2	2.6	2.4–3.2	–
16	Body: greatest width	ca. 1.8	ca. 2.4	ca. 1.8	ca. 2.8	[c]	[c]	–	2.1	1.7–2.2	1.5	3.4–4.1	2.0
17	Pectoral fin: length	[c]	6.3	6.7	6.0	6.5	5.3	–	4.5	4.8–5.2	5.5–7.1	5.3–7.0	5.4–7.4
18	Pelvic fin: length	[c]	3.5	3.7	[c]	[c]	3.1	–	2.6	2.6–3.2	4.7	4.1–4.7	2.7–3.3
19	Adipose fin: length of base	2.0	2.2	2.4	3.1	[c]	2.4	–	–	2.3–3.4	2.5	2.2–3.2	–
20	Anal fin: length of base	5.9	6.4	6.0	ca. 6.2	[c]	5.0	5.8	–	5.1–5.5	6.1–7.3	5.3–7.6	–

21	Snout tip to anterior insertion pectoral	[c]	23.3	22.9	20.8	22.8	20.5	–	21.4	23.0–24.9	24.7	21.8–25.0	20.1–20.2
22	Snout tip to anterior insertion pelvic	57.8	58.9	57.3	56.4	57.9	55.4	56.7	62.3	60.3–62.3	58.3–59.9	55.2–61.0	57.7
23	Snout tip to anterior origin adipose	91.5	90.9	90.5	89.8	[c]	91.3	–	–	91.7	–	90.0	91.2
24	Snout tip to anus[h]	59.5	61.2	60.1	58.6	[c]	[c]	–	–	62.4–63.5	–	57.8–ca. 64[i]	–
25	Pectoral base[j] to anterior insertion pelvic	[c]	33.4	30.6	34.1	33.3	33.4	–	40.9	38.8[k]	–	34.0	36.2[k]
26	Pelvic base[j] to anterior origin anal	31.2	30.1	29.8	30.8	31.0	33.8	–	24.1	26.9–29.7	28.0	27.2–31.4	31.2–33.1
27	Pelvic base[j] to midbase caudal	41.2	41.1	41.1	42.2	[c]	43.7	–	37.7	38.5–39.7	40.1	42.1–44.4	42.2–43.8
28	Anus[l] to anterior origin anal	29.9	28.6	28.0	30.2	[c]	[c]	–	–	27.3	25.6	29.1	–
29	Posterior origin adipose to midbase caudal	6.9	7.2	7.1	6.6	[c]	6.6	6.8	–	5.2–6.7	6.8–7.8	7.3–8.5	–
30	Sex	♀	♀	♀	♀	[c]	[c]	–	–	–	–	–	–
31	Egg diameter (*mm*)	0.1	0.1	0.1	0.2	–	–	–	–	–	–	–	–
32	Immature	Yes	Yes	Yes	Yes	–	–	–	–	–	–	–	–

[a]Antarctic specimen modified from Nybelin (1946) and type specimen from W of Strait of Gibraltar modified from Zugmayer (1911b) and Roule (1927).
[b]Modified from Roule (1927) and Maul (1946).
[c]Unsuitable for this measurement.
[d]Snout to end of bony operculum.
[e]Eye.
[f]Eye 2.2, 3.0 (Rofen, 1966).
[g]Least bony in Templeman's measurements and in Hubbs et al. (1953).
[h]In Templeman's measurements to midanus.
[i]Hubbs et al. (1953) 51.6 for 640 mm SL NW Pacific specimen not used. It is presumably considerably too low because the distance from snout to pelvic in this specimen is 61.0 and, judging from the other large specimen from this area in Hubbs et al., the distance from snout to anus should be about 64. This value has been used. Apart from this specimen the range is 57.8–59.7.
Posterior insertion in Templeman's measurements.
[k]Origins of both fins.
[l]Midanus in Templeman's measurements.

Meristic Characters

The *Anotopterus* specimens examined by the various authors are usually from fish stomach contents and, especially for the smaller specimens, may have the fins, particularly such delicate ones as the pelvic, injured to some degree. Roule (1935) found no pelvic fins remaining on five specimens of *Anotopterus* from albacore stomachs. The low counts of four pelvic rays by Zugmayer (1911a, b) and Roule (1927) are presumably due to injury to these fins. Zugmayer's count of 12 for the anal rays may also be low but this cannot be at all certain without examining the specimen since there is a count of 13 from a southeast Greenland specimen, which has been carefully checked by me, and a count of 13 by Roule (1927) from a specimen from Madeira. Zugmayer's caudal count of 2/16/6 is almost certainly in error.

Some caution is necessary in interpretation of vertebral and anal fin numbers. For the vertebrae, although I have included the hypural, others may have omitted it (Fig. 4). In anal fin counts, the last two rays (in one of my specimens the last three rays) are very close together and I counted them as one (Fig. 4A). An investigator has to look carefully to see these two rays and they have probably been counted as one by many authors.

From the meristic data available (Table 1) it may be concluded that, apart from the possibility of such a difference between vertebral counts of the antarctic and the North Atlantic and Pacific regions, there is no absolute difference in meristic counts between areas. The distinction between antarctic and North Atlantic specimens in vertebral number has been lessened by the count of 82 for Thurow's (1961) west Greenland specimen. Many more meristic counts are needed to detect real between-area differences. None of Zugmayer's meristic counts for his type specimen (Table 1, No. 16) of *A. pharao* are likely to be correct except for the number of pectoral rays, hence comparisons must be made with specimens of *A. pharao* from the general area of the northeast Atlantic from which the type was obtained. Within the small numbers available, the *Anotopterus* of the western North Atlantic are mostly similar to those of the eastern North Atlantic in meristic counts. An exception is the high number of 82 for Thurow's (1961) west Greenland specimen, but the other vertebral counts are not essentially different for the western and eastern North Atlantic. The new vertebral number data from Krefft (Table 1) for South Atlantic *Anotopterus* (81, 82, 83) overlap with those from the Antarctic (83, 83).

It may be concluded that the *Anotopterus* of the western North Atlantic are *A. pharao* insofar as meristic counts are concerned.

Morphometric Characteristics

Because the type of *A. pharao* was from the eastern North Atlantic, in order to show that the *Anotopterus* specimens from the western North Atlantic are *A. pharao* it is only necessary to show that they are not specifically distinct from the type. The type, however, is considerably smaller than any of the

western Atlantic specimens and measurements are lacking for many characteristics. Hence one must make allowances for differences in length and use comparisons with larger specimens from the same general area, such as Madeira, that have been recognized by many authors as *A. pharao*. Even here, morphometric comparisons are not completely satisfactory because the largest eastern Atlantic specimens measured are considerably smaller than those from the western Atlantic.

Most of the points of difference of the western North Atlantic specimens from the type — the greater orbit and distances anterior to the pelvic and the lesser distances posterior to the pelvic in the latter — are very likely due to the very much smaller size of the type. A relatively larger eye is normal in smaller fish of a species. Marshall's (1955) specimen, 100 mm, from the South Atlantic has a similarly large orbit, 2.8 SL. Changes in relative length of the anterior and posterior parts of the body, also, are not unusual with increases in size. However, in *Lepidion eques* the lengths from the snout and to the anal fin in relation to the standard length increase with increase in fish length (W. Templeman, unpublished data) the opposite of that occurring here. Also in a fish such as *Anotopterus*, which often has many breaks in the vertebral column after death and many curves so that it is sometimes almost impossible to obtain the correct length, there must be some allowance for variation between the results of individual measurers.

The greater distances from the snout to the pelvic base and to the anus in relation to the standard length in the Madeira than in the western North Atlantic specimens may very likely be explained as above because these Madeira specimens are intermediate in length between the western North Atlantic specimens and the type.

It is, therefore, concluded that, in the present stage of knowledge, the morphometric characteristics of the western North Atlantic specimens of *Anotopterus* are close enough to those of the type and of the neighbouring eastern Atlantic specimens from Madeira to be the same species.

As for the small number of morphometric differences between the western North Atlantic *Anotopterus* and those of other areas, the differences in body height are probably not important as this character is highly variable within a fish species and, owing to the condition of most of these specimens, body height measurements are not very accurate. The greater postorbital length of larger North Pacific specimens may be real, but there is overlapping for this characteristic between the smaller North Pacific and the western North Atlantic specimens. Measurements for a range of *Anotopterus* lengths from both areas are needed to clarify this matter. Meanwhile it is concluded that, on present knowledge, western Atlantic specimens of *A. pharao* are sufficiently like those of the North Pacific and Antarctic in their morphological characteristics to be the same species.

Food

The small number of food records from intermediate and adult sizes has been of pelagic fishes: Marshall (1955), the paralepid *Notolepis coatsi*; Taylor

(1959), the greenling *Hexagrammus superciliosus*; Thurow (1961), the launce *Ammodytes marinus*; Postolaky (1962), the paralepid *Paralepis coregonoides borealis*; Jow (1963), the shortbelly rockfish, *Sebastodes jordani*, and a blue rockfish, *Sebastodes mystinus*; Rofen (1966), small unidentified fishes; and Templeman, the capelin *Mallotus villosus*. Pelagic feeding on fairly large food objects is also indicated by the form of the jaws and of the teeth of *Anotopterus*.

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APPENDIX 1

RECORDS OF *ANOTOPTERUS PHARAO* FROM THE WESTERN NORTH ATLANTIC

(The data are usually in order of record number, name of authority, day, month, year of capture, position or locality of capture, depth of capture, temperature at depth of capture, standard length or total length in mm, and remarks. Except where stated otherwise each record is of only one specimen.)

1, Nybelin (1946), 27 VII 1935, 64°25′N, 53°30′W, about 700 TL, head, only, from stomach of halibut, described as *Anotopterus arcticus* n.sp. *2*, *3*, Postolaky (1962), 62°27′–62°31′N, 51°17′–51°22′W, bottom trawl, 4.5 C: VII 1959, 250–300 m, 750 TL; VIII 1959, 180–275 m, 870 TL, described as 82 and 87 cm in length in different parts of his paper, but Postolaky (personal communication, 1969) says the correct length was 87 cm. *4*, *5*, Blacker (1966), bottom trawl: V 1964, off Frederikshaab or on Dana Bank, 658 SL; IX 1964, Dana Bank, 75 m, 734 SL (lengths by Templeman from specimens in British Museum). *6*, Brandes et al. (1957), Kotthaus and Krefft (1957), 23 IX 1955, 61°46′N, 50°38′W, 200 m, 2.3 C, 80 head length, head, only, from stomach of cod, bottom trawl. *7*, Thurow (1961), 4 XI 1960, 60°25′N, 48°10′W, 250–300 m, 840 TL, bottom trawl. *8*, *9*, G. Krefft (personal communication, 1969), German trawler, bottom trawl: IV–V 1961, W. Greenland, 545 SL; 15 VII 1964, off Cape Farewell, 150–170 m, about 530 SL, in stomach

of cod. *10*, Templeman, 4 VIII 1960, 55°27′N, 56°36′W, 358 m, 1.45 C, 810 SL, from stomach of 92-cm *Anarhichas denticulatus*, *A. T. Cameron*, bottom trawl. *11*, Templeman (also Squires, MS, 1955; Fisheries Research Board of Canada, 1957), 18 IX 1954, 54°50′N, 53°29′W, 722 m, 3.93 C, about 825 SL, head only, from stomach of Greenland halibut, *Investigator II*, bottom trawl. *12*, Templeman, 17 VIII 1967, 49°15′30″N, 48°16′00″W, 2200–2250 m (temperature at 1660 m, 3.16 C, estimated temperature at 2200 m, about 3 C), about 760 SL, posterior half only, from gut of 1294+ mm TL *Hydrolagus affinis* taken on bottom longline by the *A. T. Cameron* (the identity of the *Anotopterus* was checked and the length estimated by radiographs against other specimens of this species from the Newfoundland area). *13*, Postolaky (1962), VIII 1960, 46°31′N, 44°51′W, 340–375 m, about 700 TL (not less than 700 mm, length of head of one specimen 17 cm), two heads only, from a cod stomach, bottom trawl. *14*, Popova (1962) and Popova (personal communication, 1967) 19 VII 1960, 46°30′02″N, 45°02′W, 350–375 m, 250 SL, in stomach of 62-cm cod. *15*, A. I. Postolaky (personal communication, 1968) VII 1965, 46°30′N, 44°30′W, 620 m, about 600 TL, research vessel *Neptun*, bottom trawl. *16*, Templeman (also Leim and Scott, 1966), 11 X 1952, 44°00′N, 49°05′W, 296 m, 427 SL, from cod stomach, Newfoundland trawler *Blue Foam*, bottom trawl. *17*, Templeman, ca. 1953–58, E Grand Bank, 688 SL, Newfoundland otter trawler, bottom trawl. *18*, Rofen (1966), 15 VII 1958, 40°00′N, 68°14′W, 453 SL, from stomach of *Alepisaurus*.

APPENDIX 2

Records of *Anotopterus pharao* from the Central and Eastern North Atlantic

(Records are in the same order as in Appendix 1)

1, Templeman (from British Museum), 1964, 62°N, 33°W, about 135 m, 567 SL, BMNH, 1964.12.21:9, sent to museum by D. H. Jones S.M.B.A. *2*, Haedrich (1964), 1 V 1963, 40°17′N, 36°07′W, 0–about 145 m, 277 SL, from stomach of *Alepisaurus brevirostris*, surface longline. *3–6*, Legendre (1934), Roule (1935), in Bay of Biscay, from stomach of albacore tuna: 6 VIII 1931, 180 miles SW of Penmarc'h, 137 TL; 8 VII 1933, about 80 miles SW of Concarneau, 153 TL; 21 VII 1933, 45°20′–46°40′N, 7–9°W, 151 TL; 25 VII 1933, 250 miles WSW of Penmarc'h, 158 TL. *7*, Roule (1935), 1934, Bay of Biscay, 156 TL (length from Legendre, 1934), from stomach of albacore tuna. *8*, Collett (1896), 6 VII 1888, 44°01′26″N, 15°31′W, near surface, 253 TL, misidentified as *Plagyodus* sp., from stomach of albacore tuna. *9*, Nybelin (1948), 38°25′N, 10°23′W, about 14 SL, 2-m ring net, 5000 m wire. *10*, Zugmayer (1911a, b), 4 IX 1910, 36°54′30″N (36°64′30″N in Zugmayer, 1911a, but 36°54′30″N in Sta. list, Zugmayer, 1911b), 11°49′W, 154 SL (from Hubbs et al., 1953), Bourée net, 5100–0 m (presumably 5100 m wire out). *11*, Roule (1927), I 1926, Madeira, Camara de Lobos, 271 SL (from Hubbs et al., 1953), taken on bottom longline. *12–24*, G. E. Maul (personal communication, 1967), several miles off the coast usually S of Funchal, Madeira, about 32°35′N, about 16°50′W, 40–100 m, temperature surface waters at Madeira about 17 C from January to April and about 21.5–22.5 C from August to October, >220–<342 SL for all specimens whose lengths are not stated, from stomach of *Alepisaurus ferox*, No. 12–14 included in Maul (1946): 12 IV 1943, 342 SL; 5 III 1944; III 1944, 278 SL, described in Marshall (1955); No. 15 included in Maul (1952), 28 XI 1946, 47.5 SL; about 1953, four specimens; 21 I 1957, two specimens; 11 II 1957; 9 III 1957, six specimens; 29 IV 1959; 14 IX 1959; 16 II 1961; 23 II 1962; 18 III 1965. *25*, *26*, Hubbs et al. (1953), before 1953, Madeira, obtained from G. E. Maul, Museu Funchal: 247 SL (Rofen, 1966, 244 SL for this fish); 260 SL. *27*, G. E. Maul (personal communication, 1968), 19 IX 1961, 32°24′15″N, 16°45′30″W, 2500–0 m, 759 SL, British research vessel *Discovery*, Isaacs-Kidd pelagic trawl, ripe female, Museu Funchal No. 20272. *28*, G. Krefft (personal communication, 1969), 22 I 1968, 29°23′N, 18°10′W, about 2000 m, 87.4 mm, *Walther Herwig*, pelagic trawl.

Lipids and Fatty Acids of Two Species of North Atlantic Krill (*Meganyctiphanes norvegica* and *Thysanoëssa inermis*) and Their Role in the Aquatic Food Web[1]

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The fatty acid compositions of phospholipids and triglycerides from two lots of *Meganyctiphanes norvegica* (M. Sars), and of total lipids of two additional lots of different origin, showed characteristics commonly found in lipids of carnivorous higher animals such as herring. The fatty acids in both phospholipids and triglycerides of one sample of *Thysanoëssa inermis* (Krøyer), although qualitatively similar to those of the *M. norvegica* lipids, showed a closer quantitative relationship to phytoplankter fatty acids and suggested that this species is herbivorous. These observations are discussed in terms of biological data on the feeding habits of the animals, and their basic biochemical processes as related to overwintering or other times of food scarcity. The crustacean samples, obtained from the stomachs of fin whales captured off Nova Scotia, had 1.2–2.4% total lipid, with nonsaponifiable materials accounting for 3–5% of the lipids in *M. norvegica* and 10% in *T. inermis*. The potential utilization of these species in artificial rearing of trout or salmon is discussed.

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INTRODUCTION

This study examines the lipid and fatty acid compositions of two of the larger North Atlantic zooplanktonic Crustacea, *Meganyctiphanes norvegica* (M. Sars) and *Thysanoëssa inermis* (Krøyer). These two euphausiids are plentiful in offshore Nova Scotian waters, at least in certain seasons, and virtually pure samples were obtained for examination from the stomachs of baleen whales (hence our appellation "krill"). The establishment of commercial rearing of trout and other salmonids in Nova Scotia (Anon., 1966) suggested an examination of these krill as an unexploited fishery resource. Subtle variations in the composition of body lipids of salmonid fish may have influence on flavour and keeping quality, and it is therefore important to establish the character of lipids and fatty acids in the more exotic potential

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foodstuffs. Krill have been harvested from Norwegian fjords on a limited commercial scale as a food for trout and other salmonid fish being reared for human consumption (Wiborg, 1965; Wiborg and Bjørke, 1968), and harvesting at sea of Antarctic krill (*Euphausia superba*) has been demonstrated to be practical (Anon., 1969).

Lipid studies on zooplanktonic Crustacea have concentrated on *E. superba*, but analytical data of varying degrees of complexity and completeness in respect to fatty acids and lipids have been recorded for other Crustacea as well (most pertinent references are listed and discussed by Ackman and Eaton, 1966a, 1967; see also Klem, 1932; Saiki and Mori, 1956; Saiki et al., 1959; Lasker and Theilacker, 1962; Lovern, 1964; Tsuyuki et al., 1964a, b; Williams, 1965; Jeffrey et al., 1966; Bottino et al., 1967a, b; Lewis, 1967; Arnold, MS, 1968; Raymont et al., 1968; Culkin and Morris, 1969; Pierce et al., 1969). *Meganyctiphanes norvegica* and *Thysanoëssa raschii* were reported by Fisher (1962) to show significant differences in fatty acids (see Ackman and Eaton, 1967) but this was not correlated with the proportion of triglyceride to phospholipid, which has a strong influence on compositions of total fatty acids. Exploration of these lipid classes and their fatty acid compositions was also designed to investigate the role of *M. norvegica* and *T. inermis* in the transfer of lipids and fatty acids through the aquatic food web.

EXPERIMENTAL

Sample Origin

Samples of krill taken from fin whale (*Balaenoptera physalus*) forestomachs when the whales were inspected prior to processing at New Harbour, near Blandford, N.S., were preserved in formaldehyde solution for a few days prior to removal to Halifax. The field numbers recorded for these particular whales (all adults) by M. Cawthorne of the Arctic Biological Station, Fisheries Research Board, Ste. Anne de Bellevue, P.Q., and certain other data on origin, are as follows: for *Meganyctiphanes norvegica* I, Finwhale B-167, taken October 9, 1966; II, Finwhale B-200, taken October 23, 1966; III, Finwhale B-362, taken November 24, 1967; and IV, Finwhale B-28, taken July 30, 1965 (see Ackman and Eaton, 1966a, 1967). *Thysanoëssa inermis* was obtained from Finwhale B-168, taken October 9, 1966.

The *M. norvegica* of sample III were appreciably smaller, ca. 20 mm in length, than those in the other samples (ca. 30 mm in length), but the sample was homogenous in respect to size. It is believed (E. H. Grainger, personal communication) that the smaller *M. norvegica* of sample III were subadult and likely to be prespawners, whereas the other *M. norvegica* were all large enough to be adults that had spawned at least once. The *T. inermis*, ca. 18 mm in length, were also possibly subadult.

Sample Treatment

Lipids were extracted from drained samples of *M. norvegica* and *T. inermis* by the method of Bligh and Dyer (1959). Formaldehyde media were not examined for lipid (Ackman and Eaton, 1966a). In some instances the lipids were fractionated on a gel column as described by Sipos and Ackman (1968). The usual separations of fish lipids achieved with this system were handicapped by several pigment bands that interfered with differential refractometry. Accordingly multiple fractions within each apparent lipid band were collected and examined by thin-layer chromatography (TLC). Where practical special attention was paid to obtaining pure fractions of phospholipids and triglycerides when combining fractions into those listed in Table 1, but some fractions with spots having the TLC mobility of free fatty acids (FFA) may have contained

TABLE 1. Details of lipid origin and gel chromatography fractions.

Sample	Date collected	Recovery of lipid (%)	Nonsaponifiable in lipid (%)	Lipid or fraction (with major components) examined	Lipid in fraction (%)	Fatty acid ester calculated iodine value
				Meganyctiphanes norvegica		
I	Oct. 9, 1966	1.58	4.9	Total lipid	–	134
				A_1 Phospholipid	28	233
				B_1 Triglyceride	42	124
				C_1 Free fatty acid, some triglyceride	21	148
				D_1 Free fatty acid, sterols, etc.	7	171
II	Oct. 23, 1966	1.22	–	Total lipid	–	216
				A_2 Phospholipid	15	274
				B_{2a} Triglyceride	5	167
				B_{2b} Triglyceride, some free fatty acid	5	189
				C_2 Free fatty acid, trace sterol esters	43	208
				D_2 Free fatty acids, sterols, etc.	16	202
III	Nov. 24, 1967	2.36	3.0	Total lipid	–	156
IV	July 30, 1965	2.19	4.9	Total lipid	–	138
				Thysanoëssa inermis		
	Oct. 9, 1966	1.83	10.3	Total lipid	–	182
				A_5 Phospholipid	34	194
				B_5 Triglyceride, some free fatty acid	13	88
				C_5 Free fatty acids, some sterol ester	29	148
				D_5 Free fatty acids, sterols, etc.	6	187

other materials of similar R_f value. *Meganyctiphanes norvegica* sample I gave very clean fractions A_1 and B_1. Sample II had apparently undergone severe lipid hydrolysis but also provided substantially homogeneous fractions A_2 and B_2. Although fraction D_2 showed only a single spot (for FFA) on TLC, this was diffuse and possibly not pure FFA. Samples III and IV were not examined in detail by type of lipid. The *T. inermis* sample yielded a high purity phospholipid (A_3), but the triglyceride fraction (B_3) was not entirely pure, and the TLC spot for FFA in fractions C_3 and D_3 was elongated and may have included other material.

Lipids and fractions thereof were esterified or transesterified with boron trifluoride–methanol (Morrison and Smith, 1964). When TLC indicated a substantial proportion of nonsaponifiable material in fractions or in total lipids, nonsaponifiable materials were first removed by a procedure reported by the American Oil Chemists' Society (1967). Except for *M. norvegica* sample IV, studied in part by packed column gas–liquid chromatography (GLC), the methyl esters were examined by open-tubular (capillary) GLC. Minor components were tentatively identified through separation factor or plotting procedures, partition of polyunsaturated esters into nitromethane, or by hydrogenation of samples, sometimes assisted by urea-complex treatment (Ackman and Hooper, 1968).

The identifications of the methyl esters of the monoethylenic fatty acids from the total lipid of *M. norvegica* sample IV by GLC were verified and extended by oxidative fission as described by Ackman and Castell (1966). The isoprenoid fatty acids were in most instances determined independently and diastereoisomer ratios for the phytanates studied after urea-complex treatment of isolated total saturated acid esters, or from hydrogenated ester samples as described by Ackman and Hooper (1968) and Ackman et al. (1969a).

In view of known open-tubular column losses for polyunsaturated fatty acid esters (Ackman et al., 1967), quantitation was verified in most instances by a minimum of two out of three possible approaches: (*a*) hydrogenation of methyl esters, either quantitative or qualitative, to determine individual chain lengths that could be compared with totals from the unhydrogenated methyl ester sample: (*b*) concurrent examination of the methyl ester sample on packed column GLC equipment using only flame ionization detector corrections; and (*c*) comparison of calculated and experimental iodine values, the latter being in some instances determined as hydrogen values.

Sample Degradation

The krill obtained from whale forestomachs would not be expected to undergo any more than slight digestion, as the forestomach, primarily a holding station, lacks secretory glands (Slijper, 1962). Only in sample III of *M. norvegica* was there a pronounced degree of degradation of the outer surfaces with detachments of limbs. In all other samples the animals were intact, although possibly somewhat blanched, suggesting recent collection by the whales.

It is to be expected, however, that some autolysis of the krill lipids will take place subsequent to the death of these animals themselves as well as of the whale. The hydrolysis of both phospholipids and triglycerides is indicated (Table 2) by the high proportion in the FFA fractions of typical phospholipid acids such as 20:5ω3 and 22:6ω3, and also of fatty acids typically derived from triglycerides such as 14:0, 20:1, and 22:1. Under the circumstances a general autolysis of all lipids is indicated (Addison et al., 1969). The recovered residual intact lipid fractions such as phospholipids and triglycerides will therefore still be fairly representative of those initially present. The hydrolysis process does possibly reduce lipid recoveries by weight since the fatty acids are about only two-thirds of the weight of original phospholipid. Thus the original total lipid present in *M. norvegica* sample II may have originally been about the same as sample I, and that of *T. inermis* somewhat higher than the figure given in Table 1.

Lipid Fractionation

It is believed that the triglyceride and phospholipid fractions from the gel column do not cross contaminate beyond a level of 1–2%. The tail of the triglyceride band may contain mono- and diglycerides, as well as the initial material in the free fatty acid fraction. The latter extends over a large volume of eluant and this includes the sterol and sterol ester bands, which are in themselves only moderately satisfactory as to purity. For the present study of partially hydrolysed

lipid, and from TLC evidence, it is convenient to regard this whole band as mostly free fatty acids; but it was divided into two types, the first or "C" fractions containing in all probability some mono- and diglyceride as well as a little triglyceride, and the "D" fractions, which may include small amounts of sterol esters, but in which the fatty acids are otherwise those derived from hydrolysis of phospholipid and triglyceride.

RESULTS AND DISCUSSION

Lipids

In our samples of euphausiids a gross examination showed no lipid reserves such as the well-defined globule or sac of oil observed in some smaller Crustacea. These lipids, although they may contain only small proportions of triglyceride, probably function as energy reserves, since they shrink or disappear in fasting periods (Jermolàjev, 1958; Littlepage, 1964). The oil sacs may be lost on collection or fixation (Comita et al., 1966), and may be specifically associated with herbivores (Conover and Corner, 1968). The Crustacea that lack this feature are believed to exist in times of fasting by a reduction in metabolic rate that may or may not involve lipids to some extent, by utilization of protein and carbohydrate materials, or by both. Lipids in the larger Crustacea are apparently at such low levels as to be excluded from consideration as materials for adjusting buoyancy (cf. Corner and Cowey, 1968).

Lipid recoveries (Table 1) were comparable for *M. norvegica* (1.22–2.36%) and *T. inermis* (1.83%). The nonsaponifiable content of these lipids was 3–5% for *M. norvegica* and twice as much (10.3%) for *T. inermis*. These results, and the observation that phospholipids (or free fatty acids derived largely from phospholipids, see below) account for nearly half the lipid, are sufficiently similar to literature studies on a variety of the larger (up to shrimp size) Crustacea as to warrent a clear distinction between lipids of these and of the smaller Crustacea. Before the introduction of silicic acid chromatography the separation of triglycerides and phospholipids was affected by precipitation of the phospholipids from acetone (e.g., Tsuyuki et al., 1964a, b). A number of studies using this technique have been included in the reference list in the introduction, and generally gave results comparable with more recent silicic acid chromatographic separations. The additional detail now available from the latter procedure may be exemplified by a number of analyses. Thus, from the lipids of *Neomysis integer*, a brackish-water, semiplanktonic mysid, Raymont et al. (1968) recovered lipid fractions as follows; phospholipids, 50%; triglycerides, 33%; sterols, FFA, mono-, and diglycerides, 12.5%; and sterol esters, 2.5%. These details compare more with *T. inermis* than with *M. norvegica* (cf. Table 1). Jeffrey et al. (1966) and Bottino et al. (1967a) reported that typical proportions among lipids of some antarctic Crustacea were: 5% FFA (when examined immediately after capture), 19% triglycerides, 48% complex lipids (phospholipids), 2% hydrocarbons, plus associated sterols, sterol esters, mono-, and diglycerides, etc. Maksimov (1968) also reported on antarctic krill fat. The krill, stated to be fresh-caught, presumably by net or pump, were not identified by species, but the lengths were 20–30 mm and the species

Table 2. Compositions of methyl esters derived from fatty acids of lipids and gel chromatography fractions, in weight percent, for various samples of two species of krill and of an averaged phytoplankter sample.[a]

Fatty acid	Total lipid						Triglyceride			Phospholipid			First free fatty acid			Second free fatty acid		
	M. norvegica				*T. inermis*	Phytoplankters[d]	*M. norvegica*		*T. inermis*	*M. norvegica*		*T. inermis*	*M. norvegica*		*T. inermis*	*M. norvegica*		*T. inermis*
	I[b]	II	III	IV[c]			I B_1[e]	II B_{2a}	B_5	I A_1	II A_2	A_5	I C_1	II C_2	C_5	I D_1	II D_2	D_5
Saturated acids																		
12:0	0.14	0.22	0.17	0.19	0.09	...	0.25	0.30	0.16	0.09	0.17	0.08	0.21	0.06	0.09	0.30	0.32	0.30
Iso 13:0	ND	0.04	0.02	–	ND	...	0.06	0.05	ND	ND	0.04	ND	ND	0.02	ND	ND	0.04	ND
Anteiso 13:0	ND	Trace	ND	–	ND	...	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
13:0	0.08	0.06	0.05	–	0.02	...	0.12	0.20	0.06	0.05	0.09	0.05	ND	0.04	0.04	0.10	0.23	0.15
Iso 14:0	0.87	0.09	0.05	–	0.03	...	0.12	0.15	0.08	0.02	0.05	0.03	0.09	0.04	0.09	0.05	0.11	0.07
14:0	5.91	4.63	5.79	4.82	3.77	6.8	7.71	6.16	7.43	1.24	1.11	1.77	6.09	2.86	4.01	7.88	7.37	6.41
4,8,12-TMTD	0.04	0.04	0.11	–	0.03	...	0.15	0.10	0.10	0.07	0.05	ND	ND	0.04	0.07	ND	0.19	0.05
Iso 15:0	0.26	0.29	0.37	–	0.12	...	0.35	0.55	0.40	0.16	0.05	0.08	0.43	0.26	0.13	0.42	0.39	0.12
Anteiso 15:0	0.12	0.12	0.14	–	0.04	...	0.12	0.28	0.18	0.08	0.02	0.06	0.20	0.09	0.02	0.15	0.25	0.04
15:0	0.75	0.85	0.78	0.56	0.29	...	0.73	1.22	0.45	0.26	0.28	0.40	0.86	0.81	0.24	0.97	1.34	0.25
Iso 16:0	0.17	0.16	0.09	–	0.03	...	0.24	0.28	0.15	0.07	0.06	0.09	0.18	0.18	0.26	0.23	0.22	0.16
2,6,10,14-TMPD	0.04	0.02	0.04	–	Trace	...	Trace	0.15	0.05	0.02	0.05	Trace	0.22	0.03	0.09	0.09	0.03	0.04
16:0	24.57	18.14	18.16	15.21	21.17	22.3	18.96	14.85	33.71	18.48	9.85	25.12	26.13	20.37	17.63	24.16	21.56	20.29
Iso 17:0	0.37	0.22	0.20	–	0.14	...	0.29	0.39	0.19	0.25	0.19	0.14	0.57	0.36	0.09	0.09	0.30	0.08
Anteiso 17:0	0.18	0.13	0.07	–	0.09	...	0.24	0.20	0.15	0.14	0.12	0.09	0.31	0.12	0.04	0.15	0.15	0.06
17:0	0.52	0.58	0.56	0.67	0.33	...	1.20	1.08	0.80	0.46	0.40	0.20	0.33	0.82	0.28	0.27	0.63	0.46
3,7,11,15-TMHD	0.26	0.20	0.22		0.01	...			0.05			0.03	0.13		0.07	0.04		0.05
Iso 18:0	0.31	0.46	0.31	–	0.19	...	0.35	0.59	0.19	0.29	0.24	0.23	0.15	0.61	0.10	0.23	0.44	0.06
18:0	2.34	1.10	1.56	1.27	2.34	1.2	2.37	2.17	2.91	1.49	1.48	2.51	2.09	1.68	2.44	0.88	0.75	2.29
19:0	0.04	0.05	0.02	Trace	0.04	...	0.06	0.20	0.04	0.07	0.03	0.05	0.13	0.05	0.03	0.05	0.07	0.04
20:0	0.14	0.10	0.10	0.01	0.05	...	0.12	0.25	0.05	0.10	0.16	0.06	0.08	0.08	0.05	0.03	ND	ND
Total saturated acids	36.3	27.6	29.1	22.7	28.5	30.3	33.4	29.6	46.5	23.5	14.5	31.0	38.1	28.6	25.8	36.1	34.6	30.9
Monounsaturated acids																		
14:1ω5	0.13	0.07	0.09	0.59	0.09	...	0.12	0.05	0.30	0.07	0.05	0.02	0.18	0.02	0.23	0.29	ND	0.26
15:1	ND	ND	0.05	0.20	ND	...	ND	ND	ND	0.13	0.02	ND	ND	ND	0.04	ND	ND	0.07
16:1ω9	0.70	0.37	0.40	0.98	0.46	0.3	0.60	0.62	0.09	0.21	0.33	0.12	0.55	0.31	0.28	0.76	0.63	0.44
16:1ω7	2.22	1.10	3.28	9.80	4.99	16.6	4.19	1.87	8.38	1.37	1.56	3.43	4.04	1.06	6.23	4.98	1.50	6.67
16:1ω5	0.09	0.34	0.40	1.03	0.16	...	0.35	0.54	0.09	0.09	0.11	0.01	0.61	0.31	0.19	0.51	0.50	0.19
17:1	0.18	0.51	0.58	0.24	0.12	...	0.24	0.54	0.08	0.36	0.41	0.09	0.24	0.54	0.13	0.30	0.78	0.32
18:1ω11[f] + 18:1ω9	12.95	11.63	11.90	10.34	15.35	2.8	12.33	11.73	11.65	14.11	13.73	9.56	13.43	13.05	26.88	8.96	9.17	12.38
18:1ω7	0.73	5.70	8.52	4.44	6.38	1.3	5.87	5.09	11.29	3.14	3.43	7.92	6.69	4.58	7.49	5.38	3.69	4.51
18:1ω5	0.69	0.43	0.55	0.61	0.30	...	0.59	1.06	0.45	0.67	0.25	0.28	0.69	0.91	0.25	0.66	0.34	0.20
19:1	0.20	0.31	0.15	0.09	0.05	...	0.21	0.40	0.11	0.24	0.17	0.12	0.42	0.42	0.20	0.27	0.27	0.07

16:1ω5	0.09	0.34	0.40	1.03	0.16	...	0.35	0.54	0.09	0.09	0.11	0.01	0.61	0.31	0.19	0.51	0.50	0.19
17:1	0.18	0.51	0.58	0.24	0.12	...	0.24	0.54	0.08	0.36	0.41	0.09	0.24	0.54	0.13	0.30	0.78	0.32
18:1ω11[f] + 18:1ω9	12.95	11.63	11.90	10.34	15.35	2.8	12.33	11.73	11.65	14.11	13.73	9.56	13.43	13.05	26.88	8.96	9.17	12.38
18:1ω7	2.73	4.70	5.02	4.44	6.28	1.3	5.87	5.00	11.29	3.14	3.43	7.92	6.69	4.58	7.49	5.38	3.69	4.53
18:1ω5	0.69	0.43	0.55	0.61	0.30	...	0.59	1.06	0.45	0.67	0.25	0.28	0.69	0.91	0.25	0.66	0.34	0.20
19:1	0.20	0.33	0.15	0.09	0.05	...	0.23	0.49	0.11	0.24	0.17	0.12	0.42	0.49	0.09	0.29	0.27	0.07
20:1ω11	Trace	0.18	0.13	1.12	0.25	...	0.41	0.38	0.29	0.18	0.20	0.09	} 3.24	} 2.15	0.16	0.13	0.09	} 0.48
20:1ω9	5.75	1.51	6.85	8.35	1.51	0.3	8.14	6.10	2.00	1.60	1.43	1.24			1.66	1.26	0.76	
20:1ω7	0.81	0.23	0.57	0.89	0.48	...	1.16	0.88	0.50	0.24	0.21	0.32	0.38	0.30	0.42	0.13	0.90	0.24
20:1ω5	0.12	ND	0.04	0.09	0.09	...	0.15	ND	0.04	0.05	ND	0.03	0.11	0.03	0.05	ND	ND	0.11
22:1ω13 + 11	4.02	1.07	5.61	11.61	1.45	...	6.33	5.07	1.31	0.35	0.66	0.72	1.26	1.02	0.93	0.62	0.30	0.27
22:1ω9	0.75	0.15	0.65	0.81	0.36	...	1.27	0.67	0.38	0.09	0.17	0.21	0.14	0.16	0.15	0.09	ND	0.14
22:1ω7	0.26	ND	0.07	0.64	0.16	...	0.40	ND	0.09	0.05	0.11	0.17	0.05	0.08	0.10	ND	ND	0.09
24:1ω9	0.65	0.63	ND	0.51	0.39	...	0.52	0.66	0.05	ND	ND	0.62	0.20	0.47	0.21	0.15	ND	0.14
Total monounsaturated acids	36.9	23.5	36.8	52.3	32.5	21.3	42.9	35.7	37.1	23.5	23.2	25.0	32.2	25.5	45.5	24.8	18.1	26.5
Polyunsaturated acids																		
16:2ω7	0.11	0.06	ND	ND	0.07	...	0.12	ND	0.09	0.08	ND	0.09	0.20	ND	0.06	0.06	0.12	0.13
16:2ω6	0.09	0.02	ND	ND	0.06	...	0.06	ND	0.05	0.05	ND	ND	0.11	ND	0.07	0.05	ND	0.15
16:2ω4	0.37	0.11	0.07	0.44	0.38	...	0.06	ND	0.94	0.02	0.12	0.40	0.29	0.07	0.37	0.43	0.07	0.46
16:3ω4	0.13	ND	0.24	0.37	0.03	...	0.24	ND	0.53	0.09	ND	0.21	0.09	ND	0.07	0.11	ND	0.21
16:3ω3	0.10	0.04	0.02	ND	0.26	...	ND	0.04	0.09	0.01	0.04	0.04	0.09	0.07	0.28	0.24	0.07	0.49
16:4ω3	0.06	0.04	0.13	ND	0.02	3.0	0.12	0.09	0.09	0.07	0.02	0.04	0.07	0.02	0.07	0.10	0.02	0.18
16:4ω1	0.18	ND	0.13	0.59	0.07	0.6	0.24	0.20	0.82	ND	0.06	0.15	0.20	ND	0.12	0.40	0.04	0.11
18:2ω6	1.81	2.22	1.70	1.35	1.27	3.4	1.47	1.67	1.18	2.02	2.11	1.55	1.59	2.18	1.08	1.66	2.13	0.96
18:3ω6	0.12	0.29	0.32	0.04	0.09	0.8	0.12	0.05	0.09	0.07	0.02	0.05	0.15	0.01	0.06	0.13	0.10	0.14
18:3ω3	0.85	1.04	1.15	0.44	0.78	7.9	1.02	1.12	0.70	0.75	0.71	1.07	1.04	0.91	0.57	1.30	1.25	0.73
18:4ω3	1.12	1.14	2.40	1.29	1.23	6.4	2.36	2.42	1.64	0.44	0.23	1.27	1.03	0.73	1.04	1.71	1.52	1.58
20:2ω6	0.44	0.48	0.32	0.34	0.10	...	0.40	0.38	0.26	0.75	0.52	0.46	0.24	0.49	0.10	0.19	0.25	0.27
20:3ω6	0.12	0.11	0.61	0.10	0.07	0.1	0.15	0.17	0.03	0.08	0.04	0.07	0.07	0.04	0.15	0.23	0.20	0.18
20:3ω3	0.62	0.45	0.44	} 0.56	0.17	0.1	0.46	0.37	0.09	1.05	0.89	0.22	0.48	0.75	0.25	0.03	0.54	0.11
20:4ω6	0.56	1.21	0.43		0.80	2.4	0.28	0.54	0.31	0.94	2.01	0.94	0.83	1.27	1.03	0.96	1.28	0.79
20:4ω3	0.54	0.54	0.62	0.52	0.38	0.1	0.52	0.62	0.15	0.57	0.31	0.25	0.61	0.67	0.61	0.71	0.78	0.33
20:5ω3	9.36	12.34	8.71	9.27	21.43	10.7	7.21	6.30	7.10	15.65	12.43	24.33	12.98	10.77	18.65	21.44	15.78	28.98
21:5ω2	0.22	0.14	0.25	0.19	0.28	...	0.28	0.78	0.09	0.39	0.20	0.25	0.35	0.31	0.11	0.27	0.41	0.21
22:4ω6	ND	ND	0.10	0.45	ND	...	0.02	ND	0.01	ND	ND	0.02	ND	ND	0.09	ND	ND	0.17
22:5ω6	0.17	0.27	0.21	0.17	0.09	0.2	0.10	0.07	0.01	0.42	0.42	0.11	0.16	0.27	0.04	0.11	0.66	0.12
22:5ω3	0.50	1.75	1.53	0.67	0.16	0.1	0.31	0.05	0.09	0.54	1.23	0.27	0.32	1.18	0.13	0.23	0.82	0.40
22:6ω3	10.12	26.72	13.39	8.14	10.92	4.9	8.01	19.51	1.31	28.62	40.60	12.34	8.46	26.81	3.70	8.62	21.30	5.54
Total polyunsaturated acids	26.8	49.0	34.2	24.7	39.0	40.7	23.6	34.7	16.3	53.0	62.2	44.1	29.4	46.4	28.7	39.0	47.2	42.5

[a]ND = not determined, usually because of insufficient sample in relation to GLC sensitivity.
[b]Sample (Table 1).
[c]This analysis carried out on a packed GLC column.
[d]Averaged from 12 species of unicellular algae (Ackman and Tocher, 1968), partial data only (see text).
[e]Fraction (Table 1).
[f]Always a minor isomer in proportion to 18:1ω9, but separation from 18:1ω9 in GLC analyses dependent on column efficiency. Proportion given in text for sample IV.

was more likely *Thysanoëssa* sp. than *E. superba*, which is as long as 4 cm when mature. Lipid (1.4 and 1.9% of wet weight, respectively, in two samples) had iodine value of 133, nonsaponifiables 5.3%, and FFA 23% (by titration). Culkin and Morris (1969) studied the epipelagic euphausiid *Euphausia brevis* and found lipids to be 2.1% (wet weight), reportedly mostly triglyceride, with 11.7% nonsaponifiables in the lipid. They also determined the total fatty acid composition and in addition studied six mesopelagic decapods on the same basis. Pierce et al. (1969) compared lipids and fatty acids of *E. superba* and *E. pacifica* with those of a crab, *Pleuroncodes planipes*. Extractable lipid (dry weight) was in the range 18.7–23.8% in the euphausiids, corresponding to about 1.9–2.4% wet weight. The thorough study of Arnold (MS, 1968) compared in detail lipids of four Crustacea from a Sargassum community: a small shrimp (*Latreutes* sp.), a large shrimp (*Leander tenuicornis*), a barnacle (*Lepas pectinata*), and a crab (*Portunis sayi*). The lipid contents of the two shrimp were closely comparable (10.9 and 10.2% respectively on a dry weight basis). The lipids also were fairly similar in detail with, respectively, sterols plus sterol esters at 5 and 11%, triglycerides 6 and 16%, FFA 2 and 2.5%, mono- and diglycerides totaling 10.5 and 5.7%, and polar lipids at 76.2 and 63.8%. It may be concluded from the similarity of our results to those of these studies with diverse species of larger Crustacea that, as a rule of thumb, the larger (10–50 mm total length) euphausiids and other similar Crustacea contain only modest amounts of lipid (0.5–2.0%, wet weight), with phospholipid (30–50% or more) as the principal type of lipid. Nonsaponifiables in the lipid are low (5–10%). By contrast the smaller Crustacea (< 2 mm), especially copepods, often contain relatively more lipid (< 10%, wet weight) and of this nonsaponifiable matter may be as high as 50% (Collin et al., 1934; Yamada, 1964), indicating a fundamentally different lipid composition.

It should be noted that the titration of total lipid extract does not give accurate FFA levels. Acidic phospholipids (phosphatidyl serine and phosphatidyl inositol) react with alkali at the conventional phenolphthalein end point, although a differential titration may overcome this (Ackman et al., 1969b). In view of the high proportion of phospholipid in lipids of most Crustacea (see above), and the likelihood that they contain phosphatidyl serine and inositol (Tsuyuki et al., 1964b), isolation of FFA by chromatographic means (or at least separation from phospholipids prior to titration) is necessary to produce valid results. The high FFA figures obtained by Maksimov (see above) probably result in part from titration of phospholipids, whereas Pierce et al. (1969) considered that the high FFA level in their samples, indicated by TLC, probably resulted from lipid autolysis. This process, which is normal (although delayed, even under good preservation conditions such as frozen storage (Ackman et al., 1969b)), undoubtedly accounts for the high proportion of FFA demonstrated in our krill lipid samples by chromatography. The normal level of FFA in whole living euphausiids may perhaps be 2–5%, possibly differing in composition from other lipids (Bottino et al., 1967a, compare

Addison et al., 1969), depending on the state of digestion of stomach contents, as well as on the general total lipid level. In a freshly killed shrimp (*Pandalus borealis*) that had been held without food for several days, the authors were unable to detect any FFA by TLC examination of the total lipid (Ackman and Eaton, 1967).

Basic review material on the occurrence and metabolism of lipids and fatty acids in marine Crustacea will be found in Giese (1966) and Corner and Cowey (1968). However, not all aspects of metabolism may be defined as clearly as earlier writers proposed. The functions of the two principal types of lipids in *M. norvegica* and *T. inermis*, respectively the phospholipids and triglycerides, may cautiously be segregated by analogy with higher organisms (Lovern, 1964). Phospholipids are strictly characteristic of cellular organization and a few other specialized functions or materials, such as nervous tissue, whereas triglycerides may be regarded as structural elements only when present in very low proportion, and alternatively as depot fats primarily for energy reserve only when they exceed the level of phospholipids by a factor of two or more.

It is not entirely clear whether variations in metabolism in poikilothermic organisms that have access to food in varying degrees on a year-round basis (e.g., *Neomysis integer*, Linford, 1965; Raymont et al., 1968), are really similar to those in organisms for which food is plentiful at one time of year, and relatively absent for much of the rest of the year (e.g., *Calanus finmarchicus*, Comita et al. 1966; Conover and Corner, 1968). Moreover there are indications of populations "acclimatized" to specific oceanic conditions (Matthews, 1968; see also Conover, 1961). In respect to fat in Crustacea care must be observed in comparing calorific values from organic-matter analyses vis-à-vis direct bomb calorimetry (Comita et al., 1963, 1966), as fat resists oxidation.

In Nova Scotian *M. norvegica* samples, the highest lipid levels (Table 1) are found in the July (IV) and November (III) samples. Fisher et al. (1954) indicated that "oil" (i.e. lipid) was highest (6–7%) in *M. norvegica* from Loch Fyne in July 1951, and a little lower (4–6%) in the following period October–February. Aside from one extremely low value in March ($<1\%$), the maximum "oil" value appears to be about twice the minimum. The variations in our four samples are not very extreme, if allowance is made for the lipid hydrolysis in sample II. Our maximum overall lipid values for both *M. norvegica* and *T. inermis* are generally lower than those of Fisher et al. (1954) (wet weight basis) for corresponding seasons. Possibly phytoplankters, or other food sources, are more available in Loch Fyne than in the open ocean. Other eastern Atlantic samples examined on a *dry weight* basis (a ratio of 5:1 seems reasonable for comparison of dry- and wet-weight results, although Pierce et al. (1969) suggest 10:1) showed as high as 23.5% fat (26-i-66) or 17.9% fat (4-iii-66) (Conover and Corner, 1968) or as low as 5.3% fat (9-iv-63) (Blumer et al., 1964). Since there was very little nonsaponifiable matter (Table 1) in this species compared to the high values ($\sim$50%) reported for other crustacean zooplankters, and this was made up largely of sterols sharing common R_f

values with cholesterol on TLC, the variation in lipid was presumably largely in the triglyceride level, although total phospholipid in the animal may also drop when spawning takes place since the eggs are apt to be rich in all types of lipid. Probably the permanent phospholipid level was of the order of 0.5% on a wet-weight basis, if lipid hydrolysis in sample I was not too extensive and may be taken as a guide.

Our lipid value (1.83%) for *T. inermis* in the fall corresponds to a season of fairly high fat level for *T. raschii*, as observed by Fisher et al. (1954). In the latter species the "oil" decreased gradually through the winter, but fairly substantial proportions, possibly giving a maximum (~9%) about four times the minimum (~2%), were deposited in spring and fall as phytoplankter blooms occurred. The proportion of phospholipid in whole *T. inermis*, about 0.5%, is essentially the same as that estimated for *M. norvegica*, but the level of nonsaponifiable material (mostly appearing as sterol on TLC) in the lipid is twice as great. These observations can be interpreted as indicating that our sample of *T. inermis* was not fully fattened up for the overwintering period. If *T. inermis* does store appreciable fat for overwintering, it would be expected to be triglyceride of the depot fat type, but since *M. norvegica* does not apparently lay down as great a portion of fat for winter utilization, the triglyceride could well be of a different type. It is therefore necessary to compare the fatty acids of specific lipids as well as the total fatty acid composition (see below).

Crustacean Fatty Acids

Comparison of the fatty acid compositions of *M. norvegica* and *T. inermis* (Table 2) points towards a correlation between certain types of fatty acids found in the total lipids and particular lipids, and the occurrence of similar types of fatty acids in the food intake. These particular fatty acid differences are superimposed on the basic species-oriented fatty acid patterns arising from genetic and metabolic differences.

The much-studied *E. superba* is a pure herbivore, its food collection mechanisms being particularly adapted to the collection by filtration of the diatom *Fragilariopsis antarctica*. Not many of the larger euphausiid crustaceans are so specific. It is believed on the basis of the exhaustive studies of Fisher et al. (1954) that subadult *M. norvegica* are primarily filter feeders ingesting organic detritus and perhaps unicellular algae, whereas mature *M. norvegica* are predators, feeding particularly on copepods. These observations, made on hauls from Loch Fyne, are probably applicable to pelagic specimens as well, although it should be noted that *Euphausia pacifica*, which has similar preferences in diet to *M. norvegica*, can become habituated to a particular diet and thereafter show a conditioning effect in selecting food (Lasker, 1966). Fisher et al. (1954) reported that *T. raschii* was essentially a herbivore, and this may be true of *T. inermis* as well. Although Nemoto (1967) considered *T. inermis* to be a herbivore, he stressed the necessity of detailed anatomical studies to ensure accuracy in drawing such conclusions for euphausiid feeding

patterns. An alternative to morphological considerations may be the use of metabolic data (Conover and Corner, 1968).

Of the four *M. norvegica* samples, one (sample II) was distinguished by the high iodine value of the fatty acids in toto (Table 1) as well as by details of fatty acid in the individual lipids (Table 2). Very high levels of 22:6ω3 occur in all lipids of this sample, including the triglyceride, and 20:5ω3 is also high, but not especially so, in the triglyceride in comparison with sample I. The reason for this high level of 22:6ω2 is unknown but evidently is related to the fact that this sample had the lowest lipid recovery of the four even when some allowance was made for low weight recovery due to lipid hydrolysis. Selective mobilization of fatty acids in a fasting period (Kaneko et al., 1967b) is a possibility.

The other three samples of *M. norvegica* are definitely in a different category as regards lipids and fatty acids. The much lower iodine values (Table 1) for total lipids, triglycerides, and free fatty acids (where determined) are obviously associated with the presence of substantial amounts of 20:1 (range 7–11%) and 22:1 (range 5–13%). Unexpectedly, sample IV also shows two to three times the level of 16:1 acid observed in samples I–III, and sample I has a considerably higher level of 16:0 than the others. The ubiquitous 18:1 is fairly constant, although a little higher in sample I than in samples II–IV.

Comparison of triglycerides is especially interesting since total fatty acids of *M. norvegica* samples I and II differ in certain component fatty acids. The suggestion that less "essential" fatty acids are being catabolized in sample II is supported by the relatively somewhat lower levels of 14:0, 16:0, 16:1, 20:1, and 22:1; the key acid 18:1 has not apparently been affected and the percentage of 22:6ω3 is extremely high to offset the others. The *T. inermis* triglyceride has a very low iodine value. Correspondingly it has twice as much 16:0 and 16:1ω7, and somewhat more 18:1, than the *M. norvegica* triglycerides. Most commonplace fatty acids, including 14:0 and 20:5ω3, are present in similar proportions in all three triglycerides, but the low iodine value in the *T. inermis* results from the very low proportion of 22:6ω3. The low percentages of 20:1 and 22:1 in this triglyceride, although similar to those in *M. norvegica* sample II, are of special interest. We believe that this particular pattern of monoethylenic fatty acid distribution is basically a species peculiarity, and yet also reflects the phytoplankter diet of *T. inermis*. The inclusion of an "average" phytoplankter fatty acid composition in Table 2 is based on one analysis each of 2-week-old laboratory cultures of 12 species (from 8 classes) of phytoplankters, most of which are important members of the North Atlantic phytoplankter community (Ackman et al., 1968). The fatty acid composition for *T. inermis* triglyceride shows a greater resemblance to that of the phytoplankter average than do the *M. norvegica* triglycerides in a number of points such as the low levels of 22:6ω3, 20:1, and 22:1. The higher proportion of 16:0 suggests that the immediate past dietary history of the sample may have included ingestion of *Bacillariophyceae* in which species nearly half the fatty acid may be 16:0 (Ackman et al., 1968), but this and other fatty acid details are also

apparently characteristics of *E. superba* and *E. pacifica* (Pierce et al., 1969) and therefore may have broader species-oriented implications.

Although 18:1ω7 is present together with 18:1ω9 in a number of the phytoplankters mentioned above, the proportions in the three crustacean triglycerides for the acids 16:1ω7, 18:1ω7, and 18:1ω9 suggest that exogenous 16:1ω7 is most extensively converted into 18:1ω7 by *T. inermis*. The equal proportion of these two 18:1 acids is unexpected in a marine lipid as 18:1ω7 is usually the minor 18:1 isomer by a factor of 2–10 (Ackman and Castell, 1966; Brockerhoff and Ackman, 1967; Kuemmel and Chapman, 1968).

Curiously, the exception to this is to be found in the polar lipids of jellyfish (*Cyanea capillata*). The triglycerides had a more or less normal ratio pattern of 1.1%–16:1ω7, 5.2%–18:1ω9, and 1.9%–18:1ω7, but the polar lipids had 16:1ω7 virtually lacking (0.1%), and 18:1ω9 and 18:1ω7 nominally identical at 1% each (Sipos and Ackman, 1968). It must be presumed, however, that marine invertebrates can, in common with higher life forms, synthesize 16:1ω7 and 18:1ω9 when necessary (Brockerhoff and Ackman, 1967).

Oxidative fission and detailed GLC studies of the monoethylenic fatty acids isolated from the lipids of *M. norvegica* sample IV showed, in addition to the isomers listed in Table 2, 0.1% 16:1ω3, 0.1% 18:1ω3, and 0.6% 22.1ω7. Also the 18:1 acids were shown to contain 0.6% 18:1ω11 and only 9.7% 18:1ω9, and in the C_{22} acids were found 1.7% 22:1ω13 and 9.9% 22:1ω11. The proportions among the isomers in each of the four chain lengths, including the obscure 18:1ω11, 22:1ω13, and 22:1ω11, are very similar to those found in a Pacific herring oil (Ackman and Castell, 1966). The GLC pattern (Fig. 1). also resembled that of an Atlantic cod liver oil (Ackman et al., 1967) and in

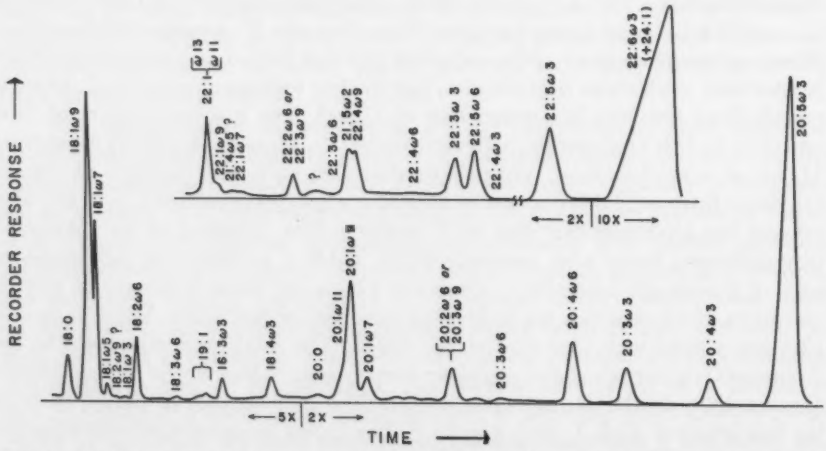

FIG. 1. Partial gas–liquid chromatographic charts from analysis of methyl esters of fatty acids from total lipids of *Meganyctiphanes norvegica* sample II. Butanediolsuccinate polyester open-tubular column, 0.25 mm I.D. × 50 m in length, operated at 170 C and 40 psig helium in a Perkin-Elmer 226. Attenuations as marked, time to 22:6ω3 approximately 1 hr.

fact is basically that common to most marine "oils." The only conclusion to be reached is that in *M. norvegica* the same processes of saturated fatty acid chain desaturation, elongation, and shortening occur as were postulated for herring and other marine vertebrates where violent depot fat fluctuations occur (Ackman and Castell, 1966). Consequently the fatty acids of *M. norvegica* should not only function as structural or metabolic elements, but also should be actively modified and altered to possess "depot" fat characteristics (including the otherwise unusual sample II) as found for herring (Ackman and Eaton, 1966b).

In *T. inermis* these processes are probably much less active, except perhaps for the conversion of 16:1ω7 to 18:1ω7, implying a basic difference in the role of the triglyceride (the high proportion of 18:1ω7 is most apparent in the triglyceride).

It is necessary to point out that the basic monoethylenic fatty acid isomer composition could be established in the smaller copepod zooplankters that both herring and *M. norvegica* prefer as food. However, in a GLC study of fatty acids of *Calanus helgolandicus* 20:1 and 22:1 are not mentioned (Lasker and Theilacker, 1962), and they are virtually absent from *Temora longicornis* lipids (Ackman and Hooper, 1970).

The phospholipids had overall fatty acid compositions generally similar to reported compositions listed elsewhere for a variety of marine species of fish and shellfish (Ackman et al., 1969b; Addison et al., 1969). Certain similarities to the triglycerides were apparent in the respective proportions of fatty acids such as 16:0, 16:1ω7, 18:1ω9, and 18:1ω7. This type of associate relationship has been noted in other marine lipids, such as moderately high levels of 20:1 and 22:1 acids in cod liver phospholipids vis-à-vis cod flesh phospholipids (Ackman et al., 1967). Presumably the phospholipids draw certain fatty acids from the same pool as the triglycerides (cf. Brockerhoff and Ackman, 1968). However, in *T. inermis* the proportions 20:5ω3 > 22:6ω3, as in the triglycerides, suggest a close relationship to phytoplankter lipids. Brockerhoff has postulated retention of β-unsaturation of plankton lipids (marked by 20:5ω3) on ingestion by higher life forms (Brockerhoff et al., 1963, 1964; Brockerhoff and Hoyle, 1967; compare Jeffrey et al., 1966; Bottino et al., 1967a, b). Examination of lipids from a variety of marine invertebrates has, in our experience, indicated that 20:5ω3 > 22:6ω3 is associated with herbivores or detritus feeders, or both, the distinction being very obscure in many instances since dietary intake is seldom accurately known in samples from nature. Moreover so little is as yet known of the movement and function of lipids in these diverse animals of various phyla that it is difficult to separate cause and effect.

Isoprenoid and other branched-chain fatty acids — The proportions of the isoprenoid fatty acids 4,8,12-trimethyltridecanoic (4,8,12-TMTD), 2,6,10,14-tetramethylpentadecanoic (pristanic), and 3,7,11,15-tetramethylhexadecanoic (phytanic) in total lipid of *M. norvegica* (Table 3) are similar to those in depot fats of higher animals such as herring and even some samples of marine

TABLE 3. Details of isoprenoid fatty acid proportions and phytanic acid diastereoisomer ratios.

Sample		Isoprenoid fatty acids as % of total	Phytanic index[a]		Phytanic acid LDD/DDD ratio
			4,8,12-TMTD	Pristanic	
Meganyctiphanes norvegica	I	0.34	12	12	–
	II	0.26	15	8	3.4
	III	0.37	30	11	3.9
Thysanoëssa inermis		0.04	300	?	2.2

[a] $100 \times \frac{\%\ 4,8,12\text{-TMTD (or pristanic)}}{\%\ \text{phytanic}}$.

mammal fats. In these crustacean lipids, phytanic acid occurs in the highest proportions of the three isoprenoid acids studied, as in the lipids of antarctic *E. superba*, a known herbivore, where phytanic acid is accompanied by very little of both pristanic acid and 4,8,12-TMTD acid (Hansen, 1969). However, in the *T. inermis* sample, phytanic, as well as pristanic acid, occurs at a lower level than 4,8,12-TMTD. It has been suggested that in higher animals accumulated phytanic and pristanic acids are partially broken down to 4,8,12-TMTD, or completely catabolized, whereas 4,8,12-TMTD is not in certain periods of fat mobilization (Ackman and Hooper, 1968). Ingestors of a diet rich in chlorophyll must have a ready means of disposing of the phytol component after it has been converted to phytanic acid; otherwise it would accumulate and possibly eventually interfere with normal lipid function. 4,8,12-TMTD (C_{16}) may be more or less harmless and participate freely in the large 14:0–16:0 fatty acid pool since the first methyl branch is the farthest removed from the carboxyl group for these three acids. In other marine invertebrates that are herbivores or at least not carnivores, 4,8,12-TMTD is the highest in proportion of the three acids (R. G. Ackman and S. N. Hooper, unpublished observations).

The diastereoisomers of phytanic acid (Table 3) are all predominantly LDD (Fig. 2). This ratio has been observed in most marine lipids and it was

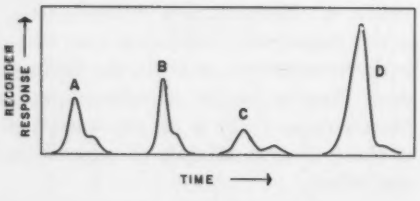

FIG. 2. Resolutions of LDD and DDD diastereoisomers of phytanic acid methyl esters. A, *Meganyctiphanes norvegica*, sample II, total lipid; B, *M. norvegica*, sample III, total lipid; C, *Thysanoëssa inermis*, triglycerides; D, R. P. Hansen preparation from antarctic *Euphausia superba* total lipid. Three different open-tubular butanediolsuccinate polyester columns, 300 ft, were used in these analyses at 150 C and 80 psig helium in a Perkin-Elmer 226.

originally proposed (Ackman and Hopper, 1968) that this enrichment was associated with stereospecific conversion of the DDD phytanic acid, or a suitable intermediate or precursor derived from phytol, to pristane, the most prominant hydrocarbon in the copepods such as *Calanus* sp. near the base

of the food web (Blumer et al., 1964). *Meganyctiphanes norvegica* could have acquired its phytanic acid from these sources, but this is not believed to be very likely in the instance of *T. inermis* and especially of *E. superba*, a known herbivore in which LDD/DDD≈18. Pristane is a very small proportion of *M. norvegica* lipids (Blumer et al., 1964), and is unlikely to be prominent in *E. superba* lipids, where nonsaponifiables are low, perhaps only 5% of total lipid (Saiki et al., 1959; Tsuyuki et al., 1964a). Tentatively it may be concluded that the phytol-pristane hypothesis to explain the high level of LDD phytanic acid, vis-à-vis the DDD isomer, is less likely than seemed reasonable on the basis of information available earlier. The ratio in favour of LDD may be associated with a basic biochemical pathway of phytol conversion or absorption of phytol or phytanic acid, or both, in all animals ingesting phytol. Unfortunately it is not possible at this time to assess the role of intestinal microorganisms in such processes in Crustacea (Baxter and Milne, 1969).

Biological oxidation of pristane can form two diastereoisomers of pristanic acid additional to the two formed more directly from phytol, and examination of L-menthyl esters in lieu of methyl esters could resolve some of these questions (compare Ackman and Hooper, 1968, and Ackman et al., 1969a).

The common *iso* and *anteiso* acids (Table 4) are of interest in that the ratios for *iso* + *anteiso* are similar to those reported for several marine life forms in a previous study (Ackman and Sipos, 1965). However, the absolute

TABLE 4. Summary of *iso* and *anteiso* fatty acid information for total lipids, triglycerides, and phospholipids.

	Total lipid				Triglyceride			Phospholipid		
	M. norvegica				*M. norvegica*			*M. norvegica*		
	I	II	III	*T. inermis*	I	II	*T. inermis*	I	II	*T. inermis*
Σ *Iso* (%)	1.18	1.26	1.04	0.51	1.41	2.01	1.01	0.79	0.63	0.67
Σ *Anteiso* (%)	0.30	0.25	0.21	0.13	0.36	0.48	0.33	0.22	0.14	0.15
Ratio $\frac{\Sigma\ Iso}{\Sigma\ Anteiso}$	3.9	5.0	5.0	3.9	3.9	4.2	3.1	3.6	4.5	4.5

percentage figures should be considered in relation to total saturated acids, in which instance they tend to be at least equal to, or generally higher than the figures reported for the higher marine life forms. In the previous study it was recognized that these acids did not likely occur in phytoplankters, as since confirmed by Ackman et al. (1968), but it was suggested that they were formed in fish. This new evidence moves their probable initial formation downwards to a more primitive organism, although quite possibly those in *M. norvegica* might be formed in yet smaller animals such as the copepods. The proportions in *T. inermis* in comparison with *M. norvegica* suggest a less

active biosynthesis, or may merely reflect the presumed herbivorous diet. The observation that monoethylenic fatty acid metabolic patterns in *M. norvegica* more closely resemble those in higher marine organisms than do those in *T. inermis* (see above) is thus extended to include the mono-methyl-branched acids. The proportions of these acids in the phospholipids in the two animals appear to be similar, and as expected lower than in triglyceride (cf. cod flesh lipid, Ackman and Sipos, 1965).

Polyunsaturated "essential" fatty acids — A much more fascinating finding in the detailed fatty acid composition data (Table 2) is the relative absence, in the krill, of the fatty acids 18:2ω6, 18:3ω6, 18:3ω3, and 18:4ω3 that were undoubtedly present in the phytoplankters at the base of the food web (Ackman et al., 1968). Some question as to the supposed inability of animals to synthesize certain of these acids has recently arisen (Rothstein and Götz, 1968), but in the present circumstances a plant origin seems assured. The transformation of the 18:3ω3 (and by inference 18:4ω3) by chain extension and dehydrogenation into 20:5ω3 and 22:6ω3 is plausible and has been the subject of experiment (reviewed by Ackman, 1967). Similarly the conversion of 18:2ω6 and 18:3ω6 into 20:4ω6, which is considered an absolutely essential fatty acid in terrestrial animal metabolism, would be plausible, but there is no large accumulation of 20:4ω6 or related materials. This suggests that these particular acids are not "essential" in primitive marine Crustacea where 20:5ω3 and 22:6ω3 are plentifully available in the diet or may be freely formed from C_{18} precursors to accommodate the lipid functions to environmental temperatures (Ackman, 1967).

The overall aspects of fatty acid compositions of *M. norvegica* and *T. inermis* are in good agreement with reliable reports for other euphausiids, and the fatty acids, in particular lipids, also conform to general aspects of marine lipid compositions in vertebrates and invertebrates. It is surprising that the total fatty acids of *Neomysis integer* (perhaps equally derived from phospholipids and triglycerides) should show exceptional amounts of 20:4ω6 (arachidonic) acid, whereas no 20:5ω3 is listed (Raymont et al., 1968). Certain details, relating to the absence of highly unsaturated polyunsaturated fatty acids in shrimp phospholipids (Arnold, MS, 1968), are questionable.

Tentative identifications have been given to most of the small components in the GLC analysis (BDS open-tubular column) of methyl esters of total fatty acid from *M. norvegica* sample II (Fig. 1). Many commonly occur in fish oils, e.g., herring oil and cod liver oil, in about the same proportions as in this sample. Among these are 20:2ω6, 20:3ω6, 20:4ω6, 20:3ω3, 20:4ω3, 21:5ω2, and 22:5ω6. In some instances a peak could be either of two acids, for example 20:2ω6 and 20:3ω9. The latter is usually associated with essential fatty acid deficiency in mammals and is less likely than 20:2ω6, unless precursor 18:2ω9 were ingested from plant sources, However, 22:3ω9 and 22:4ω9 are also possibilities where shown and could represent metabolic "dead ends" in fatty acid chain extension and dehydrogenation. The apparent presence of 22:3ω3, but not of 22:4ω6, vis-à-vis the occurrence of 20:3ω3 and 20:4ω6, suggests

that fatty acid metabolic processes in these organisms are not entirely similar to those operative in higher animals.

Extensive Japanese experience (Hashimoto et al., 1966; Hiagshi et al., 1966; Honjoh et al., 1967; Kaneko et al., 1967a, b; Watanabe et al., 1967; Sugii and Kinumaki, 1968) suggests that diets for the artificial rearing of fish require at least limited amounts of the so- called "essential" C_{18} acids, specifically 18:2ω6 and 18:3ω3, in the diet. However, it is also apparent from these studies that highly unsaturated fatty acids, such as the 18:4ω3, 20:4ω6, 20:5ω3, and 22:6ω3 that are found in *M. norvegica* and *T. inermis*, together with associated small amounts of 18:2ω6 and 18:3ω3 such as occur in these krill, would be acceptable substitutes provided sufficient tocopherol or other antioxidant were present or added to offset the deleterious effects of peroxides that might have been formed in storing or handling krill for feeding purposes. The tocopherol content of the krill is not known, but given the low lipid level of the krill and the presence of the highly unsaturated fatty acids, and carotenoids, some care to avoid prolonged storage or poor storage conditions would be necessary to promote optimum utilization of this promising natural fish food.

Potential Exploitation of Euphausiid Crustacea

The euphausiid Crustacea (or krill) contribute to the food supply of many fish species of economic importance, and are a major food of newly weaned young harp seals in the Gulf of St. Lawrence (D. E. Sergeant, personal communication). To our knowledge there has been no attempted harvesting or direct utilization by man from Canadian waters. It is known that *M. norvegica* and *T. inermis* are plentiful off Nova Scotia (M. Cawthorne, personal communication), in the western North Atlantic generally (Sergeant, 1966), and in the eastern North Atlantic (Jonsgård, 1966), since they form a very large part of the diet of baleen whales (mostly fin whales, *Balaenoptera physalus*).

Hardy (1965) reports that antarctic krill (usually understood to be *E. superba* unless otherwise identified by species) swarm in patches up to a mile in diameter that are visible to the naked eye, and that within these patches may be seen local concentrations of one to several yards across. This swarming is also reported by whalers as occurring for krill (most probably *M. norvegica*) off the coast of Nova Scotia. The "purity" of krill samples, when collected from whale stomachs, is indicative of a high degree of homogeneity in the swarms of krill. In the present study it is believed that *M. norvegica* sample I and the *T. inermis* sample were ingested within a few miles of each other, since the respective whales were killed the same day by the same boat, yet the forestomach contents, amounting to hundreds of pounds, were respectively of one species or the other. Such swarms may occur regularly, especially in the open ocean, or more erratically in sheltered waters (Forsyth and Jones, 1966; Wiborg and Bjørke, 1968). The economics of harvesting crustacean zooplankters for meal and oil production are considered to be borderline (Baalsrud, 1955; Anon., 1967). The novel food paste for human use developed

by the Russians from antarctic krill (Hardy, 1965; Anon., 1967, 1968a, b, 1969; Maksimov, 1968) would require much development work. Conversion into valuable salmonids with an established premium market value is an attractive alternative.

ADDENDUM

The publication of a definitive work on euphausiids (Mauchline and Fisher, 1969) as this manuscript went to press assembles much scattered and fragmentary information in a coherent and useful form. Among other observations it is reported that lipids in *T. inermis* range from 2.8 to 12.0% in samples from Scottish waters taken in December 1965. This reinforces the views of the authors of this work that all euphausiids, including *T. inermis*, are omnivorous and subject to highly varied and localized dietary influences. Gross compositions of individual samples need not therefore be considered representative in detail of the species. Hitherto unpublished fatty acid analyses of total lipids of *M. norvegica* and *T. raschii* are included. The *M. norvegica* composition is, overall, within the ranges reported in our tables for most important fatty acids. The *T. raschii* composition is somewhat high in total saturates (40.5%) compared to our *T. inermis* results (28.5%), but a significant point from our analysis of the latter species ($20{:}5\omega3 > 22.6\omega3$) is confirmed and may be characteristic of *Thysanoëssa* sp. The possibilities for commercial exploitation of euphausiids are reviewed.

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A Method of Surveying Scallop Populations From a Submersible

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CADDY, J. F. 1970. A method of surveying scallop populations from a submersible. J. Fish. Res. Bd. Canada 27: 535–549.

A system is described for continuously recording density changes across a population of benthic organisms from a submersible. This system was tested in the Northumberland Strait over a population of scallops (*Placopecten magellanicus*). A continuous record of scallops visible from the viewport of the submersible was registered on a chart recorder, concurrently with a series of exposures by an externally mounted automatic camera. Transects up to 4 km long were completed in a single dive.

A distance trace provided by a towed odometer wheel allowed visual counts to be expressed as linear densities (numbers seen per unit distance travelled) independently of submersible speed. An estimate of the width of visual field at a known height off bottom enabled linear densities to be converted into population densities (numbers seen per unit area of sea floor).

Scallop distribution in the area was markedly contagious. Areas of high scallop density (up to 4.8 scallops/m^2), of the order of 0.5 km across, on sand, were separated by stretches of mud where scallop density was below 0.1/m^2.

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INTRODUCTION

SEVERAL STUDIES on fisheries applications of submersibles have been published recently (Azhazha, 1961; High, 1967; Wigley, 1968; Strasburg et al., 1968; Caddy and Watson, 1969). The observations reported by these authors are largely descriptive. Attempts to estimate population densities of marine organisms from a submersible have so far involved subjective judgments of areas and distances. Any long-term use of submersibles for fisheries work must first solve the problem of making quantitative observations.

This paper describes an attempt to determine population densities of scallops in the Northumberland Strait in July 1968 from the Perry Cubmarine PC3B. The method employed during the survey should be applicable to other benthic and demersal organisms that can be observed from a submersible.

AREA OF OPERATIONS

Submersible operations were carried out on commercial scallop beds situated 10.4 km offshore from Richibucto, N.B. On July 3, concentrations of scallops were located at 46°48.2′N, 64°41.5′W, in 20 m of water on a level bottom of fine sediment. A continuously recording current meter was placed on bottom after the first dive, and seven more dives were made in relation to its marker buoy during the following week.

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EQUIPMENT AND PROCEDURES

The Perry Cubmarine PC3B, a 6.6-m long, 7-hp, two-man submersible, weighing 2497 kg, with a depth capability of 183 m, was towed to and from the work area each day by the 76-hp *Pandalus II*, a small dragger adapted for fisheries research. A 65-hp motor launch was also in attendance for sub-to-surface communication and transfer of personnel and equipment between sub and mothership.

Ancillary Equipment

A system for recording scientific observations was installed on the submersible, and had four main components: (1) An odometer wheel (Fig. 1A), towed along bottom behind the submersible, measured ground speed and distance travelled during a dive. Each wheel revolution was recorded electrically as a pulse. (2) An Edgerton, Germeshausen and Grier (EG&G) camera and flash unit (Fig. 1B), set to commence independent operation approximately 10 min after reaching bottom, took benthic photographs during the dive. A photocell inside the flash unit reflector registered each flash discharge. (3) A manual switch (Fig. 2) was depressed by the observer each time a scallop was seen through the viewport. (4) A multi-channel chart recorder, mounted in front of the observer (Fig. 3, 4), recorded wheel revolutions, photo flashes, and visual counts. The observer was able to make brief notes (dive time, course changes, etc.) directly onto the recorder paper.

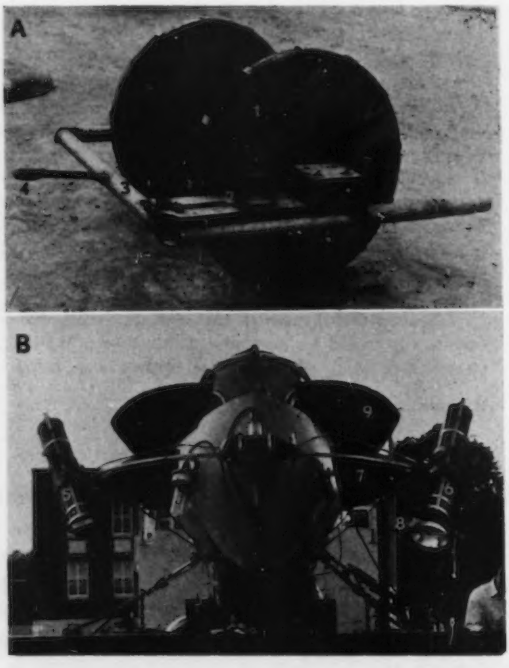

FIG. 1. Ancillary recording equipment attached externally to the Perry Cubmarine PC3B. (A) Odometer wheel seen from the side with (1) magnet; (2) reed switch embedded in plastic; (3) lead to through-hull connector on submersible; (4) towing eye. (B) Front view of submersible with (5) EG&G camera and (6) flash unit attached by brackets to the supporting frames of the forward dive planes; (7) dive planes; (8) photo cell unit wired to the inside of the flash unit reflector; (9) towing vanes (removed before dives).

Odometer

The odometer consisted of two plywood wheels (Fig. 1A) 0.61 m in diameter on an iron axle that rotated within hardwood axle blocks. The blocks were bolted onto a U-shaped framework of 5.1-cm diam iron pipe. Metal cleats at intervals around the perimeter of the wheel improved

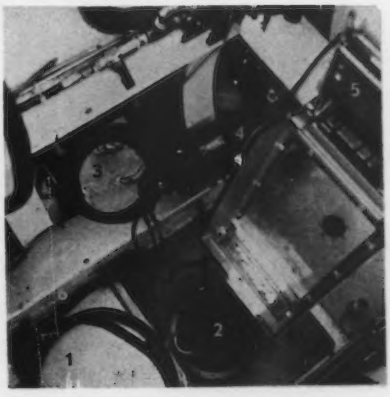

FIG. 2. Inside view of the PC3B through the rear hatch. (1) Observer seat; (2) ventral viewport; (3) viewport used for biological observation; (4) manual switch used for recording animals seen through viewport; (5) chart recorder (chart removed); (6) leads from through-hull connectors (photocell and odometer).

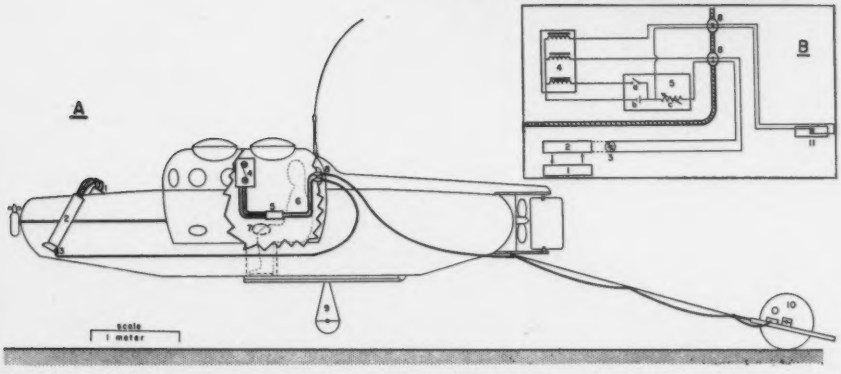

FIG. 3. (A) Diagrammatic representation of Perry Cubmarine PC3B with odometer wheel attached. (Ancillary recording systems in heavy line.) (B) Schematic circuit diagram of recording system. (1) EG&G camera; (2) flash unit; (3) photocell; (4) multi-channel clockwork chart recorder; (5) manual switch unit; (*a*) switch; (*b*) battery (2 × 22.5-v mercury cells); (*c*) variable resistance for photocell gain; (6) observer; (7) observer viewport; (8) through-hull connectors; (9) drop keel; (10) odometer wheel; (11) reed switch.

cohesion with the bottom. The wheel circumference was 1.91 m. The trailing ends of the U-frame were each filled with 3.2 kg of lead, maintaining the towing eye clear of bottom. The whole unit weighed 54 kg in air, 24 kg in water.

A circular magnet (18-kg lift), embedded in one wheel flush with its outer surface, passed within 2 cm of a magnetic reed switch on the wheel framework. Each time the magnet passed the switch, an event was registered on the chart recorder.

In preliminary safety tests from a motor launch over a rocky area, divers observed no tendency for the odometer wheel to foul on bottom. The area surveyed in the strait consisted of smooth sand in depths of 18–21 m. Divers were at hand and, in the event of an emergency, could reach the submersible by following the marker float line when summoned by underwater telephone.

During operations, the odometer was towed from a bracket on the underside of the propeller guard (Fig. 3). Adjustments of the forward dive planes were rarely required after obtaining a trim, except when the submersible changed course with respect to the bottom current. The odometer appeared to stabilize altitude control so that the vehicle travelled at a relatively constant height above bottom.

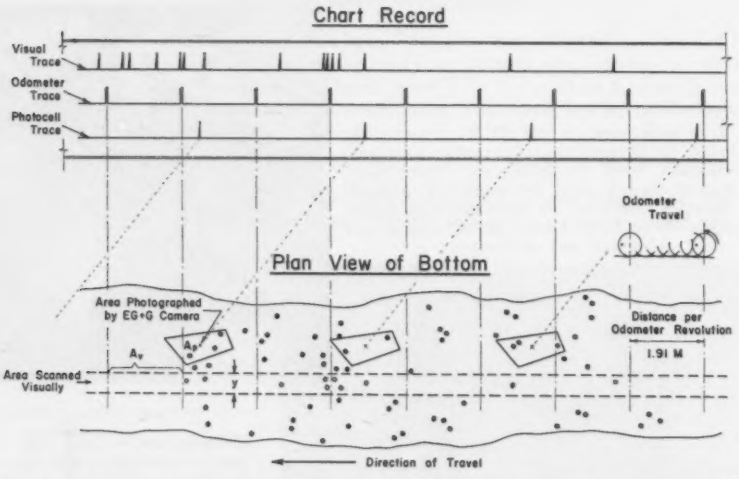

FIG. 4. Diagrammatic representation of the method of recording visual counts and camera exposures in relation to the distance travelled by the submersible.

The speed control of the submersible was maintained at its lowest point during bottom transects, and ground speed ranged between 0.35 and 0.72 m/sec, depending on heading in relation to bottom current (Table 1). Without the odometer (dive 2) submersible speed increased to above 1 m/sec, which proved too fast for satisfactory counting of benthic organisms.

CAMERA AND PHOTOCELL

The EG&G model 200 camera and its flash unit were mounted port and starboard of the nose, the camera facing forward at 33° to vertical, and inward at 20° to the center line of the submersible. A pair of quick-removal pin-and-socket brackets (Fig. 1B), on the bow-plane framework on either side, allowed a diver to remove the camera unit for reloading film and batteries. Pictures were taken automatically at 12-sec intervals until the film (500 frames) was completely exposed, or the 6-v silver-zinc batteries were discharged.

NAVIGATION

BOTTOM CURRENT SPEED

A record of bottom current speed and direction at 0.5 m above the sea floor was provided throughout the operation by a continuously recording current meter (Table 1). Bottom current speed in the area fluctuated from 0.16 to 0.31 m/sec, the strongest current running approximately north–south.

SUBMERSIBLE LOCATION

The approximate position of the submersible was known to surface personnel by reference to a large orange float towed by the PC3B. This was closely accompanied by the motor launch, which provided a radar target for *Pandalus II*. The launch was in constant communication with both the sub and *Pandalus II* by hydrophone and surface radio, respectively.

The time-consuming procedure of changing film and batteries after each dive resulted in the submersible drifting up to 1.6 km on the surface between dives. As a result, a regular search pattern could not be adhered to, since this would have required surface tows, and further reduction in dive time, already restricted by weather conditions to a few hours in the early morning.

Precise radar positioning was not satisfactory at distances over 2 km from *Pandalus II* because of the small target presented by the motor launch; so that only the initial dive position could be determined accurately. The relative positions of the dive tracks in Fig. 5B are therefore only approximate.

TABLE 1. Calculation of submersible speed and course.

Dive	Total distance travelled on bottom (*m*)[a]	Submersible heading (magnetic)	Date and time of dive-leg midpoint	Bottom current speed (*m/sec*)	Current direction (deg magnetic)	Mean distance between odometer spikes on chart (*mm*)[b]	Submersible speed (*m/sec*)	Calculated submersible course (deg magnetic)
			3/7/68					
3	1222	E	1037	0.16	310	23.7	0.37	74
		N	1109	0.16	300	22.9	0.38	339
			5/7/68					
4	2099	S	1004	0.16	10	22.2	0.40	176
5	707	N	1245	0.16	220	20.5	0.43	347
	835	W	1315	0.21	210	18.0	0.49	249
			6/7/68					
6	1597	E	1132	0.16	220	24.9	0.35	110
	294	S	1212	0.16	230	18.4	0.48	194
			8/7/68					
7	751	E	0930	0.31	360	17.5	0.50	52
	1454	N	1018	0.31	360	13.4	0.66	360
8	882	E	1151	0.26	350	16.0	0.55	62
	371	S	1212	0.21	350	20.6	0.43	185
	588	E	1221	0.21	350	14.7	0.60	70
	2086	N	1300	0.16	350	12.2	0.72	358

[a]1.91 m = 1 odometer revolution.
[b]Chart speed = 4.58 mm/sec.

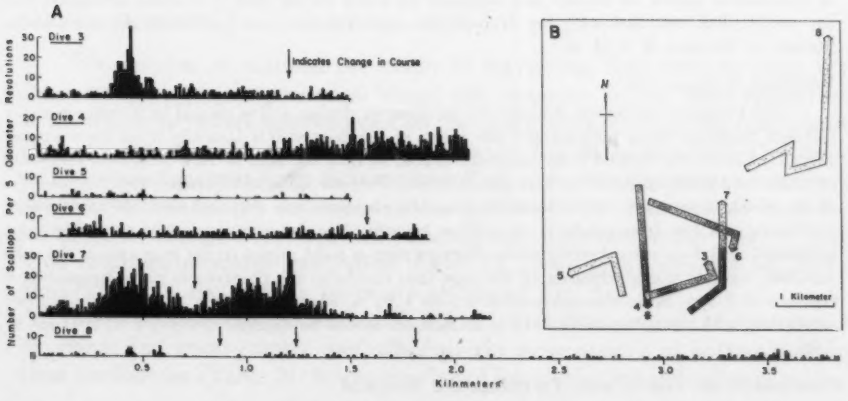

FIG. 5. Linear densities of scallops observed visually from the Perry Cubmarine PC3B off Richibucto, July 1968. (A) Detailed counts made during dives 3–8. (B) Approximate positions of the dive tracks in relation to the current meter buoy (asterisk).

An allowance for the effect of bottom current on submersible course was made subsequently from a knowledge of bottom current speed and direction (current meter), and the submersible speed (odometer + photocell traces) and heading (Table 1). This information, together with the angle of heading, allowed a corrected course to be calculated by a velocity vector triangle for each leg of the dive. Total distance travelled along bottom was given by the number of odometer spikes on the chart record (Table 1).

Underwater Visibility and Method of Observation

Underwater visibility in the area of operations dropped rapidly from about 5 m at the surface to less than 3 m below the thermocline, 3.5 m from the bottom. Low visibility near the sea floor made it necessary to stay as close to bottom as possible in order to make satisfactory counts of benthic organisms. Thirty boats dragging for scallops within 5 km of the dive area during the period of observations may have been partly responsible for the poor underwater visibility. Penetration of daylight was sufficient for counts to be made without artificial light. On July 6 (9:00 AM) an irradiance meter operated from the *Pandalus II* gave a reading of 58.3 ft-c, 0.5 m above bottom.

The lateral position of the viewport allowed little opportunity for rescrutiny of objects during the brief period they remained in sight. Errors in recognition undoubtedly occurred, especially towards the edge of the field of view. However, from previous experience during scuba dives, it was usually possible to distinguish live scallops.

Scallop counts were made with the observer looking downward and sideways through the viewport on the lower port side. The head was held at a constant distance (approximately 70 cm) away from the viewport, in order to maintain a constant field of view. Visual observations were usually continued after the EG&G film and batteries were fully discharged, although the combined effects of cold, eye fatigue, and loss of concentration usually reduced the effective observation period to less than 2 hr. At no time did dive duration approach the underwater endurance time of the PC3B (10 hr).

CALIBRATION PROCEDURES

Distance Off Bottom

An estimate of height off bottom was essential in order to convert linear counts into population densities. Although the pilot attempted to maintain the submersible at a constant height off bottom, malfunction of the depth sounder during the charter meant that the best estimate of submersible height off bottom was obtained by scuba during dive 7. A diver estimated that the submersible travelled with the drop-weight approximately one half of its diameter above bottom, a distance of 0.15 m.

Viewport Calibration

The total area of bottom scanned by an observer during a dive should be a product of the distance travelled along bottom, and the width of sea floor visible laterally from the viewport (Fig. 4). The PC3B showed little tendency to roll, so that the field of view remained relatively constant for a given height off bottom (see, however, *Deepstar*, Saila and Flowers, 1968). A measure of the width of sea floor visible laterally from the viewport was obtained with the submersible stationary and the drop-weight 0.15 m from bottom. A scuba diver stretched a marked rope graduated in 0.5-m units directly under the viewport at right angles to the long axis of the submersible. Exactly one graduation of the rope was visible to the observer in the submersible, a distance of 0.5 m. Since the submersible travels 1.91 m/wheel revolution, the area scanned per revolution, with the submersible 0.15 m off bottom, should be approximately 0.5×1.91 m^2 = 0.96 m^2.

Comparison of Visual and Photographic Records

Because camera discharges were recorded on the chart concurrently with visual counts, it was theoretically possible to establish a 1:1 correspondence between each of the photographs and the corresponding sections of the visual record on the chart. However, the area of sea floor

shown in the bottom photographs did not coincide spatially with the area scanned by eye. The camera photographed the sea floor two odometer revolutions in front, and slightly to the starboard of the area scanned simultaneously by the observer (Fig. 4). It was considered, however, that the two methods effectively sampled the same population of scallops.

The photocell trace enabled us to determine the start and finish of the photographic sequence with respect to the visual record for dives 6, 7, and 8. The mean number of scallops seen per photo ($\bar{n}_p$) and per odometer revolution ($\bar{n}_v$) during a dive could therefore be determined over the same stretch of sea floor for these three dives (Table 2). Since photographic and visual observations were made over the same population, the effective ratio of photographic to visual areas should be in the ratio of the mean number of scallops per photo ($\bar{n}_p$), to the mean number of scallops per unit of visual observation ($\bar{n}_v$). Values for the ratio $\bar{n}_p/\bar{n}_v$ (0.91 and 0.92, respectively) for dives 6 and 7 over high density areas showed good agreement (Table 2), and a value of 0.92 has been adopted in the following account.[1] A visual area of 0.96 m² and a ratio of photographic to visual areas of 0.92 suggest that the effective camera area must have been approximately 0.88 m² (Table 3).

RESULTS

Reactions of Scallops to the Submersible

Previous studies on scallops in the Northumberland Strait (Caddy, 1968) demonstrated a well-pronounced escape reaction within 0.9 m of an approaching scallop drag. A diver observing the submersible moving across a concentration of scallops noted that scallop swimming occurred only to an appreciable extent directly in front of the odometer wheel after the submersible had passed overhead. On several occasions, the observer in the submersible saw scallops swim into the field of view from astern and once, while travelling over a dense patch, tapping noises were heard from the rear, which appeared to have been caused by scallops colliding with the submersible. However, these occurrences were probably not sufficiently frequent to prejudice the estimate of population density, which does not include swimming scallops.

Linear Density

The number of scallops per meter of travel was read directly from the chart record, after comparison of visual and odometer traces. Each revolution of the odometer wheel was recorded on the chart, thus dividing the continuous visual record into a series of contiguous areas each 1.91 m long (Fig. 4). Linear densities (scallops seen/unit distance travelled) were then expressed as numbers of scallops counted per odometer revolution (Table 2). Mean linear densities for dives 3–8 ranged between 0.09 scallop/odometer revolution (dive 5) and 2.08 scallops/odometer revolution (dive 7). Continuous changes in linear density during the last six dives are shown in Fig. 5.

The frequency of occurrence of 0, 1, 2, . . . n scallops per unit of observation by visual and photographic methods over the same stretch of bottom showed close similarities (Table 2). It was concluded from this that estimates of scallop

[1]The value of 0.50 for $\bar{n}_p/\bar{n}_v$ obtained for dive 8 over a low density area was considered suspect because of the small number of scallops observed on this dive.

TABLE 2. Frequency of occurrence of scallops in visual and photographic observations over the same stretch of sea floor (dives 6–8 inclusive).

	Dive no. 6						Dive no. 7						Dive no. 8					
	Photo			Visual			Photo			Visual			Photo			Visual		
Scallops/sample	Obs[a]	Exp[a]	%	%	Obs	Exp	Obs	Exp	%	%	Obs	Exp	Obs	Exp	%	%	Obs	Exp
0	170	171.0	63.7	62.2	496	489.6	46	28.9	23.6	20.5	146	89.3	419	418.4	95.4	91.2	1784	1776.9
1	78	76.2	29.2	28.4	227	239.3	55	55.2	28.2	27.0	192	185.3	19	20.1	4.3	8.0	156	170.8
2	17	17.0[b]	6.4	7.9	63	58.5	38	52.7	19.5	19.3	137	192.2	1	0.5	0.2	0.8	16	8.3
3	1	2.5	0.4	1.4	11	9.5	22	33.5	11.3	12.9	92	132.9						0.3
4	1	0.3	0.4	0.1	1	1.2	16	16.0	8.2	9.8	70	68.9						
5						0.1	9	6.1	4.6	3.7	26	28.6						
6							5	1.9	2.6	4.4	31	9.9						
7							3	0.5	1.5	0.8	6	2.9						
8								0.1		0.7	5	0.8						
9										0.7	5	0.2						
10+							1(15)		0.5	0.1	1(10)							
No. scallops	119				390		372				1475		21				188	
No. prints	267						195						439					
No. odometer revolutions					798						711						1956	
χ^2	0.08				0.80		20.96[c]				149.70[c]		0.02				7.53[c]	
Scallops/print ($\bar{n}_p$)	0.446						1.908						0.048					
Scallops/odometer ($\bar{n}_v$)					0.489						2.075						0.096	
$\bar{n}_p/\bar{n}_v$			0.91						0.92						0.50			
Variance (V)	0.443				0.506		3.754				3.540		0.050				0.103	
Coefficient of dispersion ($V/\bar{n}$)	0.993				1.035		1.968				1.706		1.041				1.073	

[a]Obs = no. specimens observed; Exp = no. specimens expected.
[b]——— = expected frequencies below 5% pooled for chi-square.
[c]Significant difference at 5% level.

[a]Obs = no. specimens observed, Exp = expected frequencies.
[b]——— = expected frequencies below 5% pooled for chi-square.
[c]Significant difference at 5% level.

TABLE 3. Abundance of associated fauna from photographs (dives 6–8 inclusive).

Species	Dive no. 6			Dive no. 7			Dive no. 8		
	No. specimens observed	Estimated density[a] (*no./m²*)	Mean area (*m²*)	No. specimens observed	Estimated density[a] (*no./m²*)	Mean area (*m²*)	No. specimens observed	Estimated density[a] (*no./m²*)	Mean area (*m²*)
Invertebrates									
Placopecten magellanicus (Gmelin)	119	0.51	2.0	372	2.17	0.5	21	0.06	16.7
P. magellanicus (dead shell)	48	0.20	5.0	107	0.63	1.6	28	0.07	14.3
Asterias vulgaris L.	89	0.38	2.6	176	1.02	1.0	58	0.15	6.7
Echinarachnius parma (Lamarck) (live and dead)	72	0.31	3.2	118	0.69	1.5	21	0.06	16.7
Cyprina islandica L. (dead shell)	103	0.44	2.3	50	0.30	3.3	181	0.47	2.1
Polinices heros (Say)	40	0.17	5.9	6	0.03	33.3	17	0.05	20.0
P. heros (egg capsules)	8	0.03	33.3	1	0.01	100.0	42	0.11	9.1
Cancer irroratus Say	1	0.01	100.0	6	0.03	33.3	4	0.01	100.0
Buccinum undatum L.	1	0.01	100.0	2	0.01	100.0	4	0.01	100.0
Fish									
Myoxocephalus sp.	3	0.01	100.0	7	0.05	20.0	4	0.01	100.0
Pseudopleuronectes americanus (Walbaum)	4	0.02	50.0	9	0.06	16.7	2	0.01	100.0
No. frames		267			195			439	

[a]Assuming effective camera area of 0.88 m².

abundance by these two methods are directly comparable, and that, for dives 6 and 7 at least, the effective visual area (area scanned/odometer revolution) and the effective photographic coverage (area scanned/frame) were almost the same.

Population Density Estimate

In converting linear counts into population densities, the observed value for submersible height off bottom (0.15 m; dive 7) and the calculated visual area (0.96 m^2) were used to obtain estimates of mean scallop density for the areas covered by dives 6–8. Values for mean scallop density of 0.5 scallop/m^2, 2.2 scallops/m^2, and 0.1 scallop/m^2 were then calculated for these three dives. The mean density of scallops within the high density patches was 2.5 scallops/m^2, although densities of up to 7 scallops/m^2 were recorded during dive 7. (A scuba determination of scallop density in the same area in 1967 (Caddy, 1968) estimated the population density to be 1.9 scallop/$m,^2$ although maximum densities of 6.6 scallops/m^2 were recorded.)

Distribution of Scallops

LARGE-SCALE PATTERN

Superficial examination of the visual record (Fig. 5) confirmed scuba observations in 1967, which revealed marked changes in scallop density across the fishing grounds. Dives 3 and 7 passed across high density patches (4.8 scallops/m^2; dive 7), approximately 40 times the density of the surrounding areas. A gradual increase in density was observed during dive 4 along the 2.1 km of bottom traversed, from 0.5 scallop/odometer revolution to 2 scallops/odometer revolution. The distances traversed across high density patches (more than 1 scallop/odometer revolution) were estimated at 0.24 km (dive 3), 0.48 and 0.53 km (dive 7), and at least 1 km (dive 4). Although randomly positioned transects are unlikely to coincide with the diameter of an area of high concentration ("scallop bed"), these measurements give us an order of magnitude for the size of scallop beds in the Northumberland Strait.

SMALL-SCALE PATTERN

Small-scale aggregations of scallops were seen, even on the low density transects (dives 5 and 8), and prompted a comparison of visual and photographic counts with random expectation. If we assume that the submersible remains at a constant height off bottom, then the series of visual counts (scallops/odometer revolution) may be considered to represent a series of contiguous areas of equal size (A_v) (Fig. 4).

Although a series of contiguous quadrats is not randomly placed, it is permissible to test the sampled population for departures from randomness, by comparing actual counts with random expectation as predicted by the Poisson distribution, since the null hypothesis presupposes complete independence of individuals from one another (Greig-Smith, 1952).

Visual counts (scallops/odometer revolution) and photographic counts (scallops/frame) were both compared with the Poisson distribution for dives 6–8 (Table 2). The analysis was not performed over the whole dive track (Table 1) but only on those parts of the dive track for which simultaneous photographic and visual observations were available. Visual and photographic observations for dive 7 and visual observations for dive 8 showed significant departures from randomness.

One characteristic of a random distribution is that sample variance equals the mean. Departures of the variance/mean ratio (coefficient of dispersion) from unity can therefore be used to detect nonrandomness (Clapham, 1936; Salt and Hollick, 1946). The coefficient of dispersion was greater than one for all records except the photographic record for dive 6. This suggests that scallops show a contagious distribution, even at low densities (Table 2).

Associated Species and Sediment Type

One observer could record only a single species during a dive, but counts of easily recognizable demersal and epifaunal species, and dead shells on the sediment surface, were made from the benthic photographs. An effective camera field of 0.88 m^2 was considered to apply to dives 6–8 inclusive, in attempting to place an order of magnitude on faunal abundance. Faunal identification was aided by the small number of macrofaunal species and their previous occurrence in scallop dredge tows over the same area.

Eight macrofaunal species could be recognized in the bottom photographs. Counts of burrowing animals (e.g., *Echinarachnius parma* and *Polinices heros*) reflect only a proportion of the total population. Only dead shells of *Cyprina islandica* displaced to the sediment surface could be counted, but areas of maximum shell abundance seemed to coincide spatially with areas where siphon holes of living *C. islandica* were abundant. Live and dead *E. parma* could not be distinguished, so that both are included in the counts.

The bottom photographs taken during dive 7 showed a gently undulating surface, with many sand dollars and dead scallop shells and few siphon holes. Photos taken during dive 8 showed a level, fine-grain surface, perforated by numerous burrows of infaunal organisms. Egg capsules of *P. heros* and dead shells of *C. islandica* were common. Evidently, dive 7 was over sand, and dive 8 over mud: this may explain the considerable differences in scallop densities between the two dives. Dive 6 appeared transitional in most respects between the other two dives for which photographs are available.

Placopecten magellanicus was the only species recorded occurring at densities in excess of 1/m^2. Most macrofaunal invertebrates occurred at much lower densities, and their abundances were more meaningfully expressed in terms of the mean area:

$$\text{Mean area} = \frac{\text{Total area examined}}{\text{No. of individuals}} \quad \text{(Curtis and McIntosh, 1950).}$$

Most active invertebrates (*Cancer*, *Buccinum*) and fish occurred at densities of 1 or fewer/100 m^2 (Table 3). Avoidance of the submersible may have led to underestimates of the density of some motile species, but for most animals observed, a submersible presents the only direct method of obtaining an estimate of population density.

DISCUSSION

Sampling the Benthos — A Comparative Account

The development of quantitative methods for sampling the benthos has proceeded most rapidly for the infauna of particulate sediments (Holme, 1964). Most methods are modifications of the original Petersen grab, and rarely sample more than 0.5 m^2. Large epifaunal species may occur at densities much less than $1/m^2$, and are not often captured by this type of gear.

The naturalist dredge and beam trawl are still the main tools used for sampling epifauna. Attempts to quantify these essentially qualitative techniques by measuring the area of bottom scraped (e.g., the dredge with attached pedometer illustrated in Gunter (1957)) are usually unsatisfactory because of drag clogging (Cameron, 1955), mesh selection (Bourne, 1966), or active gear avoidance (Caddy, 1968). McIntyre (1956) illustrated the relative inefficiency of grabs and dredges when compared with bottom photography for estimating epifaunal density. Barnes (1955) found that dredges were less efficient than underwater television for population density estimates. Randomly placed bottom photographs were used by Dickie (1955) for assessing scallop density on the Digby scallop grounds; 143 m^2 of sea floor were photographed and 83 scallops seen during the 1950 survey; no information was obtained on the pattern of distribution. The use of photographic techniques alone may lead to problems of data interpretation, which could often be overcome if supplemented by in situ observation.

The diving "corral" devised by Scarratt (1968) and used for scallop survey work (Caddy, 1968) covered approximately 400 m^2 in 2 days and collected 750 scallops. This is undoubtedly one of the most accurate methods devised for quantitatively sampling active benthic species, but is time-consuming and arduous in operation, with a limited depth capability if work is to be carried out without decompression.

Much larger areas were sampled during the submersible survey. Assuming that the visual and photographic areas calculated for dive 7 apply to the other dives, approximately 3000 m^2 were scanned visually and 800 m^2 by the camera during the last three dives with the PC3B; 2053 and 512 scallops, respectively, were seen by the two methods. Work time was limited during this period to approximately 2 hr/day (Tyler, 1969) because of time spent under tow and freshening weather in the afternoon. Undoubtedly, a mothership with crane would have extended work time (and area surveyed) by eliminating time spent under tow.

Underwater Navigation

Accurate underwater navigation is an essential prerequisite for any systematic investigation of the large-scale pattern of a benthic organism. The method of navigation used in the present survey gave only an approximate idea of the relative positions of the six dives considered. Tracking a submersible from the surface is probably an unsatisfactory technique, especially in deeper water (Andrews, 1966). Positioning in relation to a sonic beacon on the sea floor seems more satisfactory (Emery and Ross, 1968).

Biological Pattern

Because of the extensive areas that can be surveyed from a submersible, continuous visual observation allows large-scale changes in distribution pattern to be described. Departures from randomness can be detected from random samples (e.g., Saila and Gaucher, 1966) but random sampling methods alone give little information on the scale of biological pattern. An estimate of pattern size requires some system of spaced or contiguous samples (e.g., Angel and Angel, 1967), which is difficult to achieve from a surface vessel. Undoubtedly, information of this kind could also be obtained by using a towed camera (Brundage et al., 1967), or diver-controlled photogrammetric platform (Rebikoff, 1968), but both of these methods are hampered by the surface connection; the first because of the need for cables, the second because of the limits on diver endurance imposed by the cold-water conditions of the northwest Atlantic.

Area of Bottom Visible from a Submersible

The principal weakness of the system described here is the inadequate measure of submersible height off bottom. Under ideal conditions (fixed angle of view, perfect water clarity, and freedom from pitch and roll), the areas of bottom visible from the viewport, and in the photographs, should be proportional to (height off bottom)^2.

Thus, if A_p is the area of bottom visible in each photograph, $A_p \propto B^2$ where B = vertical distance of camera lens from bottom. However, visual counts were expressed during the survey in terms of the area scanned/odometer revolution, so that a unit area of sea floor scanned by eye (A_v) equals the product of distance travelled/odometer revolution (1.91 m), and the width of the strip of sea floor scanned from the viewport (y): thus, $A_v = 1.91 \cdot y$ (Fig. 4). Since 1.91 is a constant, independent of height off bottom, $A_v \propto y$. But $y \propto C$, where C is the vertical distance of the observer's eye above bottom; therefore, $A_v \propto C$: i.e., the area of sea floor scanned visually/odometer revolution is directly proportional to submersible height off bottom.

These considerations suggest that visual surveying of the sea floor, when towing a continuously recording odometer wheel, will be much less affected by fluctuations in height off bottom, than will a photographic survey. However, monitoring changes in height from the degree of overlap of two stereoscopically mounted cameras (Brundage et al., 1967) may be more feasible than

sonar, when working close to the sea floor. The use of a "biological scale" as suggested by these authors evidently can only provide unambiguous information on the area photographed if the size variation of the species used as a biological scale is already known.

The basic assumptions of fixed viewing angle, and freedom from pitch and roll, seem substantially justified for the PC3B, and the only major limitation to the relationship proposed between visual and photographic areas is the rapid loss of resolution with distance caused by suspended material in the water (Sokolov, 1961).

Applications

With slight modification, this system of monitoring population densities could be used to determine the abundance of epifaunal invertebrates, demersal fish, and any other regularly occurring phenomenon on the sea floor. Its use for surveying the infauna is restricted to those species making characteristic burrows or other marks on the sediment. It is also restricted in its use to areas of level terrain. However, as pointed out by Thorson (1957), these level bottom areas occupy at least 90% of the sea floor.

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Relationship of Gill Ventilation and Perfusion in Pacific Dogfish, *Squalus suckleyi*[1]

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HANSON, D., AND K. JOHANSEN. 1970. Relationship of gill ventilation and perfusion in Pacific dogfish, *Squalus suckleyi*. J. Fish. Res. Bd. Canada 27: 551–564.

Unanesthetized specimens of Pacific dogfish, *Squalus suckleyi*, were used to study the relationships of oxygen uptake to ventilation and blood perfusion of the gills. Ventilation–perfusion ratios ($\dot{V}_G/\dot{Q}_T$) were determined over a wide range.

In resting dogfish the $\dot{V}_G/\dot{Q}_T$ ratios normally fell within the range of 10–20 at 6–10 C with a ventilation usually between 200 and 500 ml/min·kg and a cardiac output between 20 and 40 ml/min·kg. The extraction of oxygen from water varied from about 20 to 50% within that $\dot{V}_G/\dot{Q}_T$ range, but at lower ratios the extraction was higher and sometimes exceeded 75%. When extraction was high the oxygen tension of expired water often fell below that of arterial blood thus indicating countercurrent gas exchange.

The oxygen uptake varied almost directly with cardiac output over a wide range and with ventilation at low and normal water flows.

Data are presented regarding the effectiveness of oxygen removal from water, oxygen uptake by blood, and overall transfer of oxygen from water to blood at various ventilations.

In curarized dogfish the heart rate varied with changes of induced ventilation. The reflex nature of this cardiac and ventilatory coupling was revealed by its abolition when the fish were atropinized.

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INTRODUCTION

THE EFFECTIVENESS OF OXYGEN TRANSFER from water to blood at gills depends on many factors. The subject of the present paper is the importance of the relative flows of water (ventilation) and blood (perfusion) to oxygen uptake in unanesthetized specimens of *Squalus suckleyi*.

MATERIAL AND METHODS

Pacific dogfish, *S. suckleyi*, were caught by otter trawl in the vicinity of San Juan Island in Puget Sound. After capture they were kept in holding tanks at the Friday Harbor Laboratories or at the Seattle campus of the University of Washington.

The experimental work was done in two series. From September 1966 to May 1967, experiments were performed at Friday Harbor Laboratories with nine fish ranging in weight from 2.16

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to 4.30 kg and held at 9–10 C. Normal and subnormal ventilation volumes were employed in that series. The ventilation range was extended to higher than normal values during the second series of nine fish studied at Seattle from January to March 1969. The fish in the second series weighed 1.60–2.50 kg and were held at 6–7 C.

Surgery was performed with the fish on a special surgery table, which provided ventilation and anesthesia with M.S. 222 (tricaine methanesulfonate, Sandoz). The fish were permitted to swim unrestrained in large tanks for several hours while recovering from anesthesia. Experiments were conducted on the day of the surgery and often on following days.

Mixed venous blood was obtained by the two following methods. Usually the ventral aorta was cannulated by insertion of a flanged polyethylene catheter (PE-90) at the anterior end of the ventral aorta where the innominate arteries diverge. Cannulation did not obstruct the flow to any of the afferent branchial arteries. In a few cases surgical intervention was minimized and venous blood was obtained by percutaneous placement of a catheter into a common cardinal sinus by way of a large hypodermic needle. The needle was then withdrawn and the catheter was left in place. The two kinds of samples were not significantly different. The coeliac artery was cannulated to permit sampling of systemic arterial blood. Clotting was prevented by intravascular injection of 1000–2000 units of heparin in elasmobranch saline. Exhaled water was obtained by stitching a piece of a surgical glove over all gill slits on one or both sides such that a cutoff finger served as an exit for mixed exhaled water. The sampling catheter was mounted to draw from the center of this exhalant channel. In some specimens ultrasonic or electromagnetic blood flow transducers were implanted on the ventral aorta between the innominate and third afferent branchial arteries to provide continuous beat to beat records of pulsatile blood velocity in the ventral aorta. The anatomical arrangement precludes placement of flow transducers proximal to the origin of the most caudal set of afferent branchial arteries, thus the flow meter did not measure total cardiac output but provided an index of its relative changes.

For measurements of ventilation it was necessary to confine the fish in an experimental chamber (Fig. 1). Minimal restraint was imposed on the unanesthetized animals by means of a dam and collar through which the head was inserted. This barrier was composed of a thin rubber

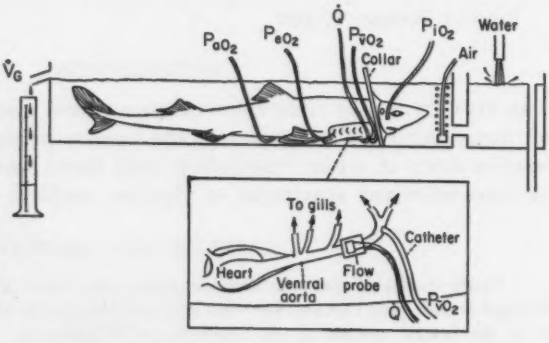

FIG. 1. Chamber for measuring ventilation ($\dot{V}_G$). A watertight dam and collar prevent water passage to the left except past gills. A flow probe permits measurement of blood flow ($\dot{Q}$), and catheters are used to sample inspired water, mixed expired water, arterial blood, and mixed venous blood (PiO_2, PeO_2, PaO_2, and $P\bar{v}O_2$, respectively). *Insert*: Flow probe and catheter positions in relation to ventral aorta.

membrane (a piece of weather balloon or rubber glove) mounted in a wooden frame. The barrier separated water bathing the anterior part of the head, including the mouth and spiracles, from the water expired from the gill slits and allowed direct volumetric measurements of the ventilation by an overflow arrangement (Fig. 1). The rubber collar was carefully fitted for each fish so it exerted only a slight pressure on the skin and appeared to have little influence on the respiratory movements. The ventilation chamber was tilted to alter the water level and, hence, the pressure gradient from the inspiratory to the expiratory sides of the barrier through a range from +5 to –35 mm of sea water. Ventilation volumes were thus increased or decreased relative to those measured normally with an inspiratory to expiratory chamber difference of less than –5 mm of sea water. These ventilation values (200–500 ml/min·kg), which we consider to be normal, are

similar to those reported earlier by Lenfant and Johansen (1966) using a similar method, and by Robin et al. (1964) using a dye dilution technique.

Blood and water gas tensions and ventilation data permitted calculation of cardiac output (Fick principle). The methods allowed comparison of ventilation–perfusion ratios over a wide range but ventilation was limited by the voluntary water pumping movements of the fish. After experiments involving normal fish were completed, the respiratory movements of the fish were stopped by intravascular injections of tubocurarine chloride (0.5–2.0 mg/kg). The mouth, spiracles, and gill slits then remained open and the experiments were repeated for comparison with the data from the normal fish.

Oxygen tensions of blood and water were measured with a Beckman 160 gas analyzer and a Beckman macro-electrode mounted in a micro-cuvette. Oxygen uptake was calculated from the measured values of ventilation, the O_2 extraction from the water, and the solubility of oxygen in the sea water. Blood flow was measured by means of a Ward 1501 ultrasonic frequency shift flow meter or a Micron RC-1000 electromagnetic flow meter.

Information on oxyhemoglobin dissociation in *S. suckleyi* (Lenfant and Johansen, 1966) allowed computation of blood oxygen saturation and content.

SYMBOLS

The following symbols are used:

$\dot{V}_G$ Ventilation or volume flow of water past gills per unit time

$\dot{Q}_T$ Volume flow of blood through gills per unit time; perfusion or cardiac output

$\dot{V}O_2$ Oxygen uptake per unit time

PiO_2 Partial pressure of O_2 in inspired water

PeO_2 Partial pressure of O_2 in mixed expired water

PaO_2 Partial pressure of O_2 in blood leaving the gills; arterial

$P\bar{v}O_2$ Partial pressure of O_2 in blood entering the gills; venous

Extr. Percentage of O_2 extracted from water passing the gills.

Ew Effectiveness of O_2 removal from water, $Ew = Extr. \frac{PiO_2}{PiO_2 - P\bar{v}O_2}$

Eb Effectiveness of O_2 uptake by blood, $Eb = \frac{\dot{Q}_T (Ca - Cv) \times 100}{\dot{Q}_T (Ci - Cv)}$ in which Ca, Cv, and Ci refer respectively to O_2 contents of blood leaving gills, blood entering the gills, and blood equilibrated to the oxygen tension of inspired water

TO_2 Oxygen transfer factor, $TO_2 = \frac{\dot{V}O_2}{\frac{1}{2}(PiO_2 + PeO_2) - \frac{1}{2}(PaO_2 + P\bar{v}O_2)}$

The expressions Ew and TO_2 are from Randall et al. (1967) and Eb from Piiper and Baumgarten-Schumann (1968).

RESULTS

Ventilation was varied through a range from 51 to 500 ml/min·kg in the first series (Table 1) and from 30 to 1310 ml/min·kg in the second series (Table 2). Low oxygen tensions of expired water and consequent high O_2 extraction values were evident at low ventilations. With increasing ventilation the extraction diminished as indicated by the higher O_2 tensions of expired water (Table 1, 2). There was no evident correlation of $P\bar{v}O_2$ with ventilation. The mean O_2 tensions of venous blood were from 10 to 13 mm Hg. Since the PiO_2 and $P\bar{v}O_2$ remained reasonably constant, the percentage effectiveness of oxygen removal from water was about 1.07 × Extr. in the second series and slightly higher in the first. The oxygen tensions of arterial blood usually

TABLE 1. Representative respiratory and circulatory parameters of nine dogfish (2.16–4.

Animal identification	$\dot{V}_G$ (*ml/min·kg*)	Oxygen tensions (*mm Hg*)				Extr. (%)	PiO_2–PaO_2	PaO_2–PeO_2
		PiO_2	PeO_2	PaO_2	$P\bar{v}O_2$			
Low $\dot{V}_G$; less than 200 ml/min·kg								
1	51	140	46	38	11	67	102	−8
2	60	132	36	73	9	73	59	+37
2	79	138	50	86	7	64	52	+36
3	81	142	75	72	3	47	70	−3
4	90	135	33	44	8	76	91	+11
5	100	138	54	88	11	61	50	+34
6	112	135	52	76	17	61	59	+24
5	135	132	57	72	7	57	60	+15
7	146	132	85	84	5	36	48	−1
6	170	135	76	87	17	42	48	+11
Mean±1 SD	102±20	136±2	56±9	72±10	10±3	58±7	64±10	16±8
Normal $\dot{V}_G$; 200–500 ml/min·kg								
8	210	130	66	88	16	49	42	+22
6	226	135	101	90	15	33	45	−11
6	256	136	74	67	14	46	69	−7
8	280	130	83	102	14	36	28	+19
9	315	104	72	57	8	31	47	−15
9	500	104	78	56	8	25	48	−22
Mean±1 SD	299±63	123±5	79±7	77±5	13±1	37±4	47±3	2±7

remained over 85 mm Hg (more than 90% saturation) at normal and high ventilations but diminished at low ventilations or when the oxygen tension of inspired water was low. Similarly, the effectiveness of oxygen uptake by blood was not less than 85% at ventilations above 200 ml/min·kg in the experiments at 6–7 C but often fell below this value at lower ventilations (Table 2). More variation was evident in the earlier experiments at 10 C (Table 1).

When ventilation increased, the increases of oxygen uptake and cardiac output maintained a nearly constant proportion in which the cardiac output was equal to roughly 40 × oxygen uptake with both expressed as ml/min·kg (Table 1, 2; Fig. 2). Since the oxygen uptake was used to calculate cardiac output these results reflect the relative constancy of the O_2 contents of arterial and venous bloods.

The effectiveness of oxygen transfer across the gills is indicated by the transfer factor. Low TO_2 values were apparent largely at ventilations below 200 ml/min·kg. There was no obvious effect of temperature on the oxygen transfer factor within the narrow range of temperatures used in this investigation.

Comparisons of curarized and noncurarized *S. suckleyi* at 6–7 C revealed that both the extraction of oxygen from water and the oxygen uptake were lower in the curarized fish at comparable ventilations (Fig. 3). Extraction

(2.16–4.3 kg) at low and normal ventilations at 10 C. Symbols defined in Materials and Methods section.

PaO_2-PeO_2	$\frac{PiO_2+PeO_2}{2}-\frac{PaO_2+P\bar{v}O_2}{2}$	Ew (%)	Eb (%)	$\dot{V}O_2$ (ml/min·kg)	$\dot{Q}_T$ (ml/min·kg)	$\dot{V}_G/\dot{Q}_T$	TO_2 $\left(\frac{ml/min}{mm\ Hg \cdot kg}\right)$
−8	69	73	69	0.19	9	6	0.0028
+37	43	78	88	0.23	8	7	0.0053
+36	48	67	94	0.28	9	9	0.0058
−3	71	48	87	0.22	6	14	0.0031
+11	59	81	67	0.37	17	5	0.0063
+34	47	66	90	0.33	12	8	0.0070
+24	47	70	85	0.37	17	7	0.0079
+15	55	64	86	0.41	14	10	0.0075
−1	58	37	92	0.27	8	18	0.0047
+11	54	48	88	0.46	20	9	0.0085
16±8	55±5	63±7	85±6	0.31±0.04	12±2	9±3	0.0059±0.0010
+22	46	56	90	0.55	23	9	0.0120
−11	66	37	91	0.41	17	13	0.0062
−7	65	51	83	0.63	27	9	0.0097
+19	49	40	94	0.53	20	14	0.0108
−15	56	34	80	0.41	15	20	0.0073
−22	59	27	77	0.52	21	25	0.0088
2±7	57±4	41±5	86±2	0.51±0.04	21±2	15±3	0.0091±0.0010

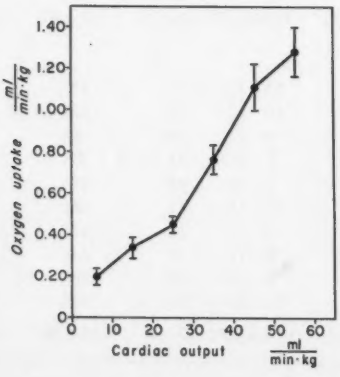

FIG. 2. Increase of oxygen uptake with increased cardiac output as measured by Fick principle in 18 dogfish at 6–10 C (means ± 1 SD). From left, numbers of measurements were 42, 61, 23, 27, 19, 17, and 7.

of oxygen from water declined steeply with increasing ventilation up to about 400 ml/min·kg in both curarized and noncurarized fish. Conversely, the oxygen uptake increased almost linearly with increasing ventilation to 400 ml/min·kg in the curarized dogfish and 500 ml/min·kg in the normal subjects (Fig. 3). At higher ventilation volumes the oxygen uptake remained almost constant as the percent extraction fell gradually.

TABLE 2. Representative respiratory and circulatory parameters of nine dogfish (1.6–2.5 k

Animal identification	$\dot{V}_G$ (*ml/min·kg*)	Oxygen tensions (*mm Hg*)				Extr. (%)	PiO_2–PaO_2	PaO_2–PeO_2
		PiO_2	PeO_2	PaO_2	$P\bar{v}O_2$			
Low $\dot{V}_G$; less than 200 ml/min·kg								
A	30	137	60	54	7	57	83	−6
B	40	149	37	49	7	75	100	12
A	50	137	74	54	9	46	83	−20
C	60	144	65	67	24	55	77	2
D	75	148	37	32	9	75	116	−5
B	90	149	29	36	7	81	113	7
A	100	137	52	60	13	62	77	8
D	125	146	33	38	7	77	108	5
E	150	149	95	95	8	36	54	0
D	170	145	62	40	12	57	105	−22
Mean±1 SD	89±24	144±2	54±11	53±12	10±4	62±7	92±9	−1±6
Normal $\dot{V}_G$; 200–500 ml/min·kg								
E	200	149	106	97	9	29	52	−9
B	210	149	49	55	7	67	94	6
E	260	150	106	76	9	29	74	−30
H	300	144	124	109	10	14	35	−15
D	340	146	82	80	9	44	66	−2
I	350	137	109	74	16	20	63	−35
D	370	149	96	80	13	36	69	−16
C	400	145	94	101	11	35	44	7
A	400	139	91	78	9	35	48	−13
D	440	148	75	75	11	49	73	0
Mean±1 SD	327±39	146±3	93±13	83±9	10±2	36±10	62±9	−11±
High $\dot{V}_G$; more than 500 ml/min·kg								
H	570	140	124	89	13	19	51	−35
F	580	137	118	74	16	14	63	−44
E	625	150	123	100	9	18	50	−23
A	650	137	90	78	7	34	59	−12
E	780	150	127	101	9	15	49	−26
C	780	144	114	110	13	21	34	−4
H	1090	147	127	104	9	14	43	−23
H	1130	147	133	104	9	10	43	−29
E	1150	150	128	94	9	15	56	−34
C	1310	146	127	110	16	13	36	−17
Mean±1 SD	867±98	145±2	121±8	96±6	11±4	17±4	48±5	−25±

When resting dogfish were permitted to respire within the normal range ($\dot{V}_G$ = 200–500 ml/min·kg), the mean ratio of ventilation to perfusion was about 15:1 at 10 C (Table 1) and somewhat lower (12:1) at 6–7 C (Table 2).

A positive gradient of mean oxygen tensions between arterial blood and exhaled water prevailed at ventilation–perfusion ratios below 15 at 10 C (Fig. 4). The gradient is accentuated at low ventilation–perfusion ratios. The

at low, normal, and high ventilations at 6–7 C. Symbols defined in Materials and Methods section.

PaO_2–PeO_2	$\frac{PiO_2+PeO_2}{2}-\frac{PaO_2+P\bar{v}O_2}{2}$	Ew (%)	Eb (%)	$\dot{V}O_2$ (*ml/min·kg*)	$\dot{Q}_T$ (*ml/min·kg*)	$\dot{V}_G/\dot{Q}_T$	TO_2 $\left(\frac{ml/min}{mm\ Hg\cdot kg}\right)$
−6	68	60	78	0.11	4	8	0.0016
12	65	78	73	0.20	8	5	0.0031
−20	74	50	76	0.16	7	7	0.0022
2	59	66	76	0.23	19	3	0.0039
−5	72	80	51	0.39	24	3	0.0054
7	68	85	60	0.50	24	4	0.0074
8	59	68	79	0.42	19	5	0.0071
5	68	81	62	0.66	32	4	0.0097
0	71	38	95	0.38	17	9	0.0054
−22	77	62	58	0.66	39	4	0.0086
−1±6	68±4	67±8	71±7	0.37±0.10	19±7	5±1	0.0054±0.0014
−9	75	31	94	0.39	19	11	0.0052
6	68	70	79	0.92	33	6	0.0135
−30	86	31	89	0.52	26	10	0.0060
−15	75	15	96	0.29	12	25	0.0039
−2	70	47	90	1.02	36	9	0.0146
−35	78	23	85	0.48	22	16	0.0062
−16	76	39	89	0.91	36	10	0.0120
7	64	48	95	0.97	51	8	0.0152
−13	72	37	90	0.90	31	13	0.0125
0	69	53	87	1.48	56	8	0.0214
−11±8	73±3	39±10	89±3	0.79±0.18	32±8	12±3	0.0111±0.0027
−35	81	21	96	0.75	34	17	0.0093
−44	83	18	85	0.55	25	23	0.0066
−23	82	19	94	0.78	38	16	0.0095
−12	71	36	90	1.46	47	14	0.0206
−26	84	16	95	0.81	39	20	0.0096
−4	68	23	96	1.14	52	15	0.0168
−23	81	15	96	1.06	43	25	0.0131
−29	84	11	96	0.79	32	35	0.0094
−34	88	16	94	1.15	52	22	0.0131
−17	74	15	95	1.18	59	22	0.0159
−25±7	80±3	19±5	94±2	0.97±0.12	42±5	21±4	0.0124±0.0021

positive gradient indicates that countercurrent exchange occurs between blood and water at the gills. In the experiments at 6–7 C the mean values for PaO_2 and PeO_2 remained negative although a positive gradient was evident in half of the experiments involving low ventilation volumes.

Both the extraction of oxygen from water and the effectiveness of oxygen removal from water decreased as the ventilation–perfusion ratio increased

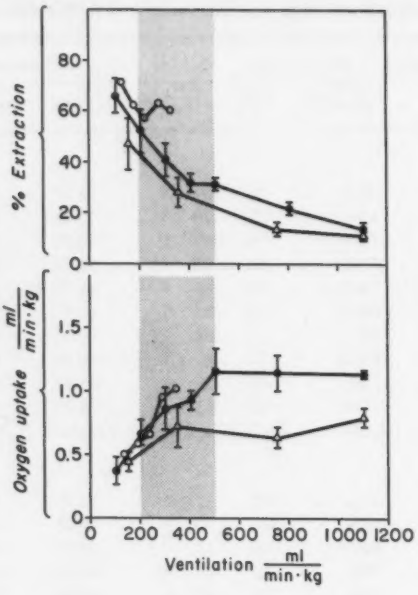

FIG. 3. Relationships of ventilation to oxygen extraction from water and to oxygen uptake in normal (solid circles) and curarized (triangles) dogfish at 6–7 C. Included are data from Baumgarten-Schumann and Piiper (1968) for *Scyliorhinus stellaris* at 16 C (open circles). Normal resting ventilation range is stippled. Values are means ± 1 SD. From the left, the numbers of measurements were 12, 12, 4, and 7 (triangles); 41, 31 13, 9, 6, 7, and 6 (solid circles).

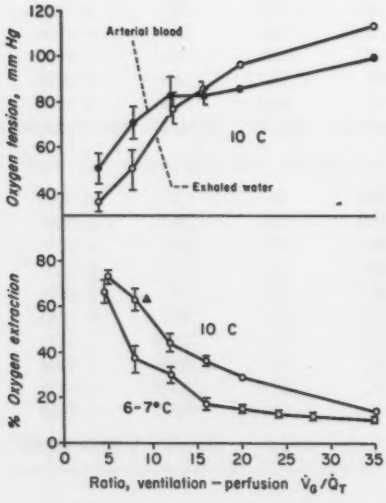

FIG. 4. Increase of oxygen tensions of exhaled water (open circles) and arterial blood (solid circles) and decrease of oxygen extraction from water with increases of ventilation–perfusion ratios. Gas tensions and extractions are shown for nine *Squalus suckleyi* at 10 C and extractions alone are shown for nine *S. suckleyi* at 6–7 C in the present study and for *Scyliorhinus stellaris* at 16 C from work by Baumgarten-Schumann and Piiper (1968) at 16 C (triangle) (means ± 1 SD). From the left, the number of measurements were 25, 26, 14, 12, 7, 4, 4, and 4 at 6–7 C; 14, 26, 17, 10, 1, and 1 at 10 C.

throughout the range of ratios determined in the present investigation (Fig. 4, 5). The extraction increased with increased temperature at constant ventilation–perfusion ratios (Fig. 4).

Curarization prevented respiratory movements by dogfish and permitted a free flow of water past the gills. In curarized dogfish the heart rate was in-

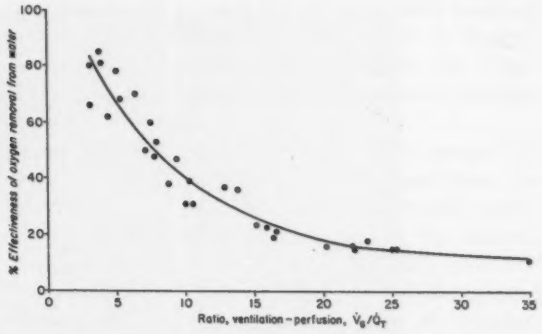

FIG. 5. Decreasing percent effectiveness of oxygen removal from water with increasing ventilation–perfusion ratios in nine dogfish at 6–7 C.

fluenced by changes of ventilation volume (Fig. 6). When water flow past the gills was reduced or stopped, the heart rate diminished and, conversely, when the water flow was restored the heart rate increased. Atropinization blocked this coupling between ventilation and heart rate.

DISCUSSION

The importance of ventilation and circulation in fish gills for their efficiency in gas exchange has been the subject of several recent publications (Hughes and Shelton, 1962; Lenfant and Johansen, 1966; Schumann and Piiper, 1966; Piiper and Schumann, 1967; Randall et al., 1967; Piiper and Baumgarten-Schumann, 1968).

The present paper provides additional information on how the relationship between ventilation and perfusion affects the oxygen uptake in the gills of the Pacific dogfish, *S. suckleyi*. The work was conducted on fish that had recovered from anesthesia and were under conditions as close to normal as feasible. In addition, an effort was made to study parameters of gas exchange with ventilation altered outside the normal range in order to elucidate factors controlling ventilation and perfusion in the normal animal.

With increasing ventilation in the normal ventilation range, which we consider to be 200–500 ml/min·kg, the oxygen uptake tends to increase despite a reduced extraction of oxygen from the water (Fig. 3). At abnormally high ventilation levels the oxygen uptake remained little changed as the oxygen extraction continued to decline. Our data parallel those of Baumgarten-Schumann and Piiper (1968) within the narrower range of ventilation values reported in their study using *Scyliorhinus stellaris* at 16 C.

In attempts to determine the optimal ventilation values for maximum effective oxygen uptake, the increased energy cost of breathing at higher ventilation values must be considered. Schumann and Piiper (1966) provide evidence that with increasing ventilation in the teleost *Tinca tinca* the oxygen requirement for breathing movements increases more rapidly than the oxygen uptake for other body functions. The present results indicate that optimal ventilation levels are below 500 ml/min·kg since higher ventilation produced

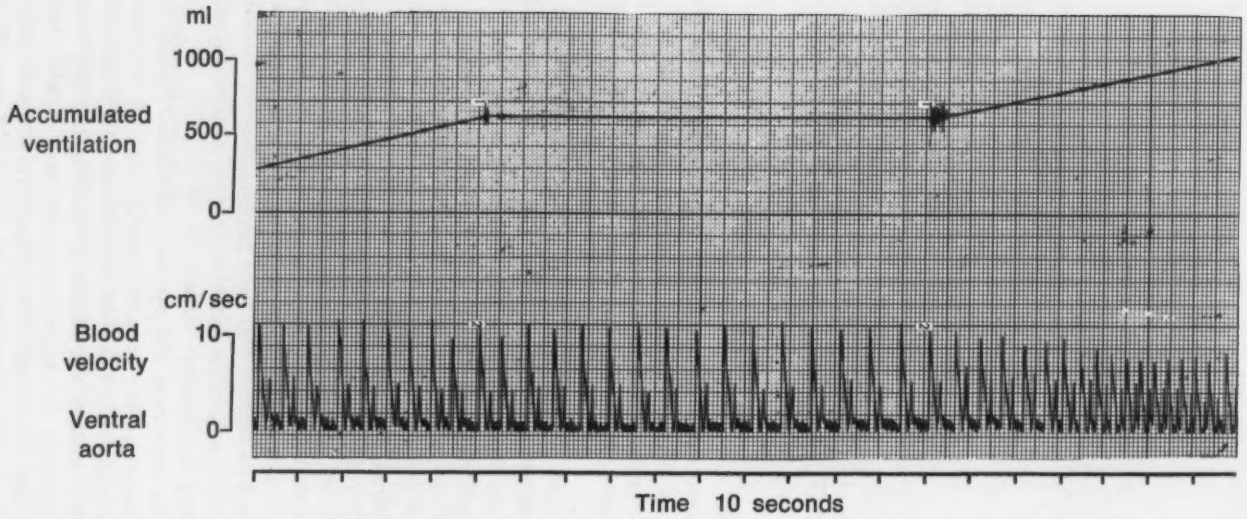

FIG. 6. Effects of ventilation changes on the heart rate and blood flow in the ventral aorta of curarized *Squalus suckleyi* at 7 C. Curarization stopped respiratory movements and left the mouth and spiracles open. Position of ultrasonic frequency shift flow probe is shown in Fig. 1.

little increase of oxygen uptake (Fig. 3). Piiper and Schumann (1967) reported an average ventilation of 248 ± 82 ml/min·kg for the dogfish *S. stellaris* at 17 C and 195 ± 55 ml/min·kg at 16 C (Baumgarten-Schumann and Piiper 1968). Robin et al. (1964) reported 450 ml/min·kg for *Squalus acanthias* at 18 C.

At subnormal ventilation values the extraction of O_2 from water was high and sometimes exceeded 75%. The effectiveness of O_2 removal from the water at low ventilation ranged from about 37 to 81% with means near 65%. Within a normal ventilation range, the percent effectiveness of O_2 removal from water was reduced to about 40% with ranges from 15 to 53% at 6–7 C and from 27 to 56% at 10 C. Piiper and Baumgarten-Schumann (1968) reported average values of 52% for anesthetized *S. stellaris*, and 56% for the unanesthetized fish. Considering the higher temperature and the relatively low ventilation volumes employed, these values seem quite compatible with the present results. Data from Randall et al. (1967) show values ranging from 11 to 30% effectiveness of O_2 removal from water by unanesthetized rainbow trout. The present results show an exponential increase of effectiveness of O_2 removal from water at decreasing ventilation–perfusion ratios (Fig. 5). It seems likely that this relationship prevails in other fish and that much of the variation reported for effectiveness of oxygen removal from water is related to differences of ventilation–perfusion ratios.

At normal ventilation values the effectiveness of O_2 uptake by blood exceeded 80% in almost all cases. The effectiveness was reduced at low ventilation values but was more closely correlated with low ventilation–perfusion ratios. Piiper and Baumgarten-Schumann (1968) reported average effectiveness of O_2 uptake by blood as 88% for anesthetized and 79% for unanesthetized specimens of *Scyliorhinus*. Randall et al. (1967), working with free swimming trout, showed higher values (95–100%) for effectiveness of blood oxygenation. Moderate exercise or exposure to hypoxic water produced a reduction in effectiveness of O_2 uptake in the trout. A similar trend seems to be indicated in the present results in which one animal was subjected to moderate hypoxia (Table 1, fish 9).

The ventilation–perfusion ratios ranged between 6 and 25 within the range of ventilations that we consider normal (Table 1, 2). Data from Baumgarten-Schumann and Piiper (1968) show a ventilation–perfusion ratio of about 9 for *S. stellaris* with the ventilation near 200 ml/min·kg. Robin et al. (1964), working with *S. acanthias*, reported data that yield a ventilation–perfusion ratio of about 18 at ventilation levels about 450 ml/min·kg. The increase of cardiac output is not in direct proportion to increasing ventilation, thus it is evident that the ventilation–perfusion ratio tends to rise as ventilation is increased.

It should be reemphasized that ventilation was altered artificially in our study and that the fish were at rest. The data, hence, do not reflect the complex adjustments of cardiac output and increased ventilation in a free swimming fish. Rather, the data provide information about the obligate

coupling of two fluid pumps in fish in which the changes of cardiac output associated with body movements have been minimized.

In the present investigation, normal values for cardiac output averaged 21 ml/min·kg in the early series and 32 ml/min·kg in the later experiments using fish of smaller average size. Robin et al. (1966), using a dye dilution technique on *S. acanthias*, reported an average cardiac output of about 25 ml/min·kg. A similar value was reported by Piiper and Schumann (1967) for *Scyliorhinus*.

A measure of general effectiveness of gas exchange is expressed by the transfer factor (T), which was used in the analysis by Randall et al. (1967) for trout and Piiper and Baumgarten-Schumann (1968) for *Scyliorhinus*. When ventilation and the oxygen tensions of inspired water and venous blood are constant the transfer factor for oxygen (TO_2) depends on the effective area available for gas exchange, the diffusion distance, and the barrier between the exchanging media. If the effective exchange area were constant, the transfer factor would provide an index of the changes of permeability or diffusibility at the respiratory exchange membranes. It seems more likely that changes of the effective exchange area with alterations of the pattern of gill perfusion would be responsible for most of the changes of TO_2.

In spite of the lack of specificity of the transfer factor, the values may provide useful comparisons. In the TO_2 equation the mean difference of O_2 tensions of water and blood is a measure of the gradient or "driving force" of O_2 between them. In our 1969 series the normal gradient averaged 73 mm Hg, which is almost identical with the average value of 74 mm Hg noted by Piiper and Baumgarten-Schumann (1968) for *Scyliorhinus*. In our earlier series of experiments the aeration of water was not quite so complete as in the 1969 series. However, consequent reduction of the O_2 driving force was accompanied by a reduction of the oxygen uptake, thus the transfer factors differed little in the two series.

The mean TO_2 figures calculated in our study were less than half the values for resting trout in water with normal O_2 tensions (Randall et al., 1967). It is expected that the gill surface area is greater in relation to body weight and the diffusion distance smaller in the gills of trout, which are more active fish with a higher resting oxygen uptake than dogfish. The smaller size of the trout may have accentuated the difference. In the present investigation the TO_2 values diminished when ventilation was low but they were not markedly influenced by abnormally high ventilation or by the difference of temperature encountered in these experiments. The TO_2 of 0.0087 $\frac{\text{ml/min}}{\text{mm Hg·kg}}$ reported for unanesthetized *S. stellaris* by Piiper and Baumgarten-Schumann (1968) is about what would be expected when ventilation is in the lower half of the normal range (Table 2).

The unidirectional flow of water past fish gills and the structural arrangement of the blood and water channels at the secondary lamellae should permit highly efficient countercurrent gas exchange between water and blood. It

has been surmised on the basis of indirect evidence that countercurrent exchange indeed occurs at fish gills. The best evidence of this exchange has been based on comparisons of gas composition in efferent branchial blood and expired water. Studies of both teleosts and elasmobranchs (Steen and Kruysse, 1964; Hanson and Martin, 1967; Baumgarten-Schumann and Piiper, 1968) have indeed shown arterial O_2 tensions exceeding those of expired water. Shunts of either water or blood at the gills could, however, mask the evidence of countercurrent exchange and result in a negative gradient of O_2 tensions between mixed arterial blood and mixed expired water. Lenfant and Johansen (1966) did not provide evidence of countercurrent exchange in *S. suckleyi* by comparison of gas tensions, but results obtained in the work by Hanson and Martin (1967) and in the present investigation leave little doubt that such exchange occurs in this species. The method used by Lenfant and Johansen for cannulating an afferent branchial vessel involved obstruction of blood flow to one or two of the gills. This or the maintenance of a relatively high ventilation–perfusion ratio may have caused the negative gradient. Non-occlusive cannulation of the ventral aorta in the present study provides unequivocal evidence of positive O_2 gradients between blood and exhaled water. A positive $PaO_2 - PeO_2$ gradient prevailed within the low range of ventilation–perfusion ratios in the experiments at 10 C (Fig. 4). The reversal of the gradient at higher ventilation–perfusion ratios may well be caused by inequalities of the distribution of blood and water at the gill exchange area or by an increase of the water dead space by mechanical separation of the gill filaments at high water flows.

Piiper and Schumann (1967) contended that the structure of elasmobranch gills precludes direct countercurrent exchange since expired water and arterial blood would flow concurrently along the interbranchial canal and thus reduce the arterial-expired gradient established in the secondary lamellae. As an alternative they suggested a multi-capillary model similar to the exchange system described for the mammalian placenta (Bartels and Moll, 1964). Extensive observation of gross and histological preparations of elasmobranch gills leads us to believe that the dimensions of the interbranchial canals and the probable flow rate of water through them would be incompatible with significant exchange between expired water and efferent blood. The fact that some arterial O_2 tensions approach those of inspired water gives support to this belief.

The principal point raised in this paper concerns the influence of ventilation and gill perfusion on effectiveness of gas exchange in *S. suckleyi*. A corollary observation is the reflex interrelation of ventilation and perfusion in dogfish (Fig. 6). Branchiocardiac coordination in elasmobranch fishes was noted by Lyon (1926) and by Lutz (1930). The reflex nature of this relationship has been elucidated in a series of papers by Satchell (1960, 1968) and Satchell and Way (1962). Their principal findings were that pharyngeal proprioceptors sensitive to stretch or pressure reflexly affect cardiac rhythms by altering the tonic activity in the cardiac branches of vagus nerves. Bursts of activity in

the pharyngeal proprioceptors occur with the branchial respiratory movements. These bursts tend to permit a release from tonic vagal inhibition such that the heart beats in a certain phase of the respiratory cycle (Satchell and Way, 1962). Our experiments on curarized, resting fish with complete paralysis of respiratory movements extend these observations by showing that alterations of ventilation independent of active respiratory movements reflexly alter the cardiac rhythm. Atropinization resulted in complete abolition of the coupling. The stimulus might be the pressure or stretch exerted by the respiratory water current on pharyngeal receptors, or the differential pressures between the oro- and para-branchial compartments even when respiratory waterflow is continuous and nonpulsatile. Our experiments do not indicate whether the reflex is identical to the branchiocardiac coupling described by Satchell, differing only in the nature of the stimulus, or whether different receptors receptive to waterflow may exist. It can be surmised that such receptors would serve an important function in coupling of ventilation and perfusion in actively swimming sharks when a large portion of the ventilation results from passive water movement across the gills in proportion to the swimming speed and the extent to which the mouth is open.

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An Electrophysiological Basis for Olfactory Discrimination in Homing Salmon: A Review

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Past and current researches relating to olfactory acuity and discrimination in fishes, with special reference to homing salmon, are reviewed.

When the nasal sac of spawning Pacific salmon is stimulated with water from the spawning site a high amplitude electroencephalographic response of characteristic pattern is recorded from the olfactory bulb. This electrical response is specific in the sense that it cannot be evoked by water from spawning sites of other groups of breeding salmon. Further, the salmon respond clearly to water taken from places along their migratory routes below the spawning sites. These findings suggest that olfaction is an important factor in guidance during the final phases of homeward migration of salmon. It is also possible that salmon retrace sequentially a trail of stimuli that is the reverse of that *imprinted* in the young fish on their seaward migration.

Although the available data do not delineate the sensitive period, or the duration of the imprinting process, there is accumulating evidence that only a short period is necessary for imprinting, which may occur when the smolts are in their freshwater life.

Recent study on the effect of antimetabolites (puromycin, actinomycin D, or cycloheximide) on olfactory bulbar discrimination in homing salmon suggests that long-term olfactory memory in these fish depends upon continued metabolism of RNA and continued protein synthesis. The possibility that the imprinting process in young fish may be affected by polluted water, which has recently become a serious problem in fisheries, is discussed. The need for electrophysiological as well as biochemical studies at a macromolecular level of the imprinting process is emphasized.

Finally, the hypothesis is discussed that a home stream odour may act most effectively as a simple "sign stimulus," which, through the release of a positive rheotropic response, induces the fish to move upstream toward home. This is largely based on the recent experimental observations of the orientation mechanism in several species of teleost fishes.

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INTRODUCTION

ALTHOUGH A NUMBER of detailed and comprehensive reviews have dealt with the olfactory cues and the mechanism of homing in spawning salmon, these have been mainly based on behavioural investigations (Hasler, 1954, 1957, 1960, 1966; Brett and Groot, 1963; Wright, 1964; Harden Jones, 1968). During the last 5 years, however, the number of studies using electrophysiological techniques to deal with the chemical sense in fish has markedly increased, thus adding a new dimension to understanding the mechanisms underlying fish migration.

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In discussing the homing mechanism of salmon, one must distinguish between the open sea navigation and the migration upstream, since the physical and physiological conditions are largely different in each system, and there is evidence that different sensory cues may play important roles in upstream migration and ocean navigation of the adult. These have often been discussed together, consequently leading to some misunderstanding.

The object of this article is to review past and current research on the olfactory response in fish with special reference to the homing of salmon and to mention a few problems that should be considered for future studies.

EVIDENCE FOR HOMING

Salmon usually return from the sea to spawn in precisely the stream or tributary in which they were reared. Normally, the place where the eggs are laid is the same as the place where they are hatched, but the two are not necessarily the same. In 1949, a large number of eggs of sockeye salmon (*Oncorhynchus nerka*) were collected on the spawning grounds of the Horsefly River, British Columbia, and transported to a fish hatchery on Horsefly Lake where they were allowed to hatch and pass their 1st year. These two places are in entirely different watersheds, separated by a range of mountains. In 1950, 94,000 fingerlings, of which 64,500 were marked, from Horsefly Lake hatchery were flown to the mouth of the Horsefly River, where it empties into Quesnel Lake, and released. In the subsequent years, a total of 282 mature sockeye salmon, of which 212 were marked, returned to the hatchery. No sockeye salmon had previously been taken at this location. On the other hand, at the spawning grounds only 1 out of 111,829 had the hatchery mark (c.f., Wright, 1964; Harden Jones, 1968).

This finding was later confirmed by Donaldson and Allen (1957) in the State of Washington, USA. They transferred coho salmon (*Oncorhynchus kisutch*) fingerlings, hatched in early 1951 and reared until January 1952 at the Soos Creek Hatchery, to both the State of Washington salmon hatchery on Issaquah Creek and to the ponds of the University of Washington College of Fisheries (Fig. 1). The fish were marked and released about 2 months after transplantation. The marked salmon returned, as adults, to the release points, but none of the marked fish returned to Soos Creek.

These results clearly indicate that the salmon do not return to the stream to which their parents returned, but to the stream where they spent their presmolting period. The period seems to be quite critical. In the case of the sockeye salmon, the critical period was presumably over by the time they were released at Horsefly River and they returned instead to the Horsefly Lake Hatchery. In the case of the coho salmon, the critical period seems to have been during or after their transplantation to Issaquah Creek Hatchery and University of Washington College of Fisheries Hatchery, so that they homed to these places.

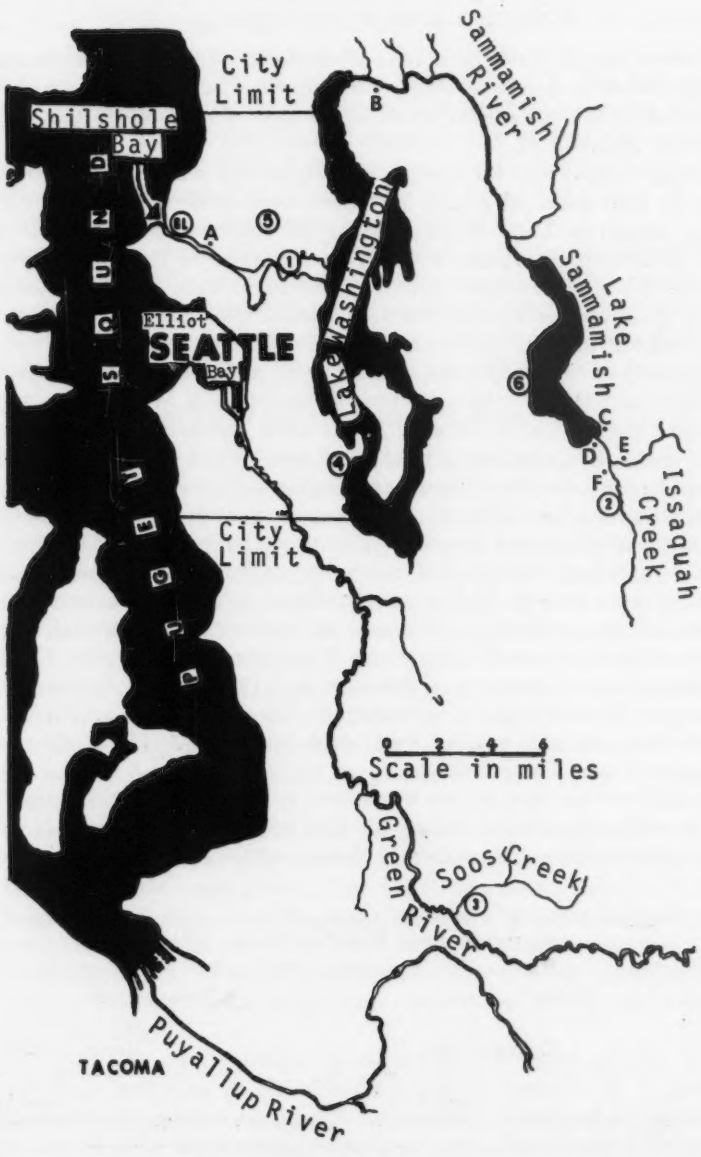

FIG. 1. Seattle and vicinity, showing the spawning sites where the fishes and waters were obtained. (1) University of Washington College of Fisheries; (2) State of Washington salmon hatchery on Issaquah Creek; (3) State of Washington salmon hatchery on Soos Creek; (4) State of Washington trout hatchery in Seward Park; (5) Green Lake; (6) Lake Sammamish; (GL) Government locks connecting freshwater level with tidal seawater level (from Ueda et al., 1967).

OLFACTORY CUES IN MIGRATING SALMON

Many theories have been offered to explain the cues and mechanisms guiding salmon to their spawning grounds during the final freshwater journey. The few experimental studies made so far have demonstrated that olfactory perception is involved.

Craigie (1926) was the first to investigate the sense of smell in guiding salmon to their natal tributary. He tagged and released about 500 migrating sockeye salmon in Deep Water Bay on the east side of Vancouver Island. In half he severed the olfactory nerve; the remainder he left intact. The elimination of the olfactory sense definitely interfered with the migration of the salmon. However, as Craigie noted, variables inherent in the experimental design made it difficult to determine whether the interference truly resulted from removal of the guiding impulses or from some other indirect effect.

Wisby and Hasler (1954) working in the State of Washington, captured returning coho salmon (*O. kisutch*) in the main Issaquah Creek and its East Fork (Fig. 1). The olfactory pits of about one-half of the fish were occluded by plugging with vaseline, benzocaine ointment, or cotton, or in some cases a combination of these. They were then replaced in the Issaquah about 1 mile below the critical stream junction. The intact control salmon showed good ability in repeating their original choice. According to Wisby and Hasler the fish whose noses were plugged were not able to differentiate between the two streams and were claimed to distribute themselves in an essentially random manner, allowing for easier entry into the larger stream (Table 1). Recent electrophysiological works by Oshima et al. (1969c) seem to support this. They captured coho salmon returning to Issaquah Creek and found that their olfactory response to East Fork water was 63.3 ± 4% of the response to Issaquah Creek (home) water.

Recently it has been shown by Groves et al. (1968) that olfaction is the major sensory mechanism directing the final migratory movements in chinook salmon (*Oncorhynchus tshawytscha*). Vision, although necessary, is less es-

TABLE 1. Recaptures (%) of control and experimental coho salmon in the Issaquah Creek experiments (from Wisby and Hasler, 1954).

Control			Nose plugged		
	Recapture site			Recapture site	
Capture site	Issaquah Cr.	East Fork, Issaquah Cr.	Capture site	Issaquah Cr.	East Fork, Issaquah Cr.
Issaquah Cr. (46 fish)	100	0	Issaquah Cr. (51 fish)	77	23
East Fork, Issaquah Cr. (27 fish)	29	71	East Fork, Issaquah Cr. (19 fish)	84	16

sential. They treated male fish to occlude their olfactory or visual senses or both, and released them upstream and downstream from the Spring Creek hatchery on the lower Columbia River, Washington. Of 866 fish released, 348 were recovered; 176 returned to Spring Creek. Three percent of the olfactory occluded, 23% of the visually occluded, and 46% of the control fish returned to Spring Creek. Of the fish recovered elsewhere, 77% were recovered at hatcheries and spawn-taking sites along the lower Columbia; 23% were recovered from sources unrelated to spawn taking.

Working with chum salmon (*Oncorhynchus keta*) in the Otsuchi River in northern Japan, Hiyama et al. (1967) have reported similar results. Thus it seems clear that the olfactory sense may play an important role in orienting adult spawning salmon, though these views have been criticized by some investigators (Ramsey, 1961; Brett and Groot, 1963).

ELECTROENCEPHALOGRAPHIC ACTIVITY OF HOMING SALMON

The first recordings of spontaneous electrical potentials (electroencephalogram, EEG) from the teleost brain were made by Adrian and Buytendijk (1931), who used isolated brain stem preparations from the goldfish (*Carassius auratus*). They recorded slow potential changes (1–3 cycle/sec) from the medulla oblongata, corresponding to the rhythm of the respiratory activity. Using implanted electrodes, Enger (1957) recorded EEG in the cod (*Gadus callarias*) when the fish was awake and when under narcosis; he also investigated the effects on EEG of photic and acoustic stimulation. Schadé and Weiler (1959) recorded characteristic patterns of spontaneous electrical activity from telencephalon, mesencephalon, cerebellum, and medulla oblongata of the goldfish using bipolar surface electrodes. They analyzed EEG responses to photic stimulation, and described localized projection of retinal areas to the contralateral optic tectum.

Electroencephalograms were first recorded from several different parts of the brain of adult spawning salmon (*O. tshawytscha* and *O. kisutch*) when they arrived at their home pond at the University of Washington College of Fisheries (Fig. 1; Hara et al., 1965). Activities in the olfactory bulb (frequency, 7–9 cycles/sec; amplitude, 35–65 $\mu\bar{V}$) and in the cerebellum (frequency, 10–12 cycles/sec; amplitude, 25–40 $\mu\bar{V}$) consistently had a much higher amplitude than those of the other parts of the brain; amplitudes of the optic lobes were especially low (Fig. 2).

In contrast to adult salmon, yearling hatchery-reared salmon generally exhibited high activities in the olfactory bulb (frequency, 8–10 cycles/sec; amplitude, 35–65 $\mu\bar{V}$) and in the optic lobe (frequency, 9–10 cycles/sec; amplitude, 25–50 $\mu\bar{V}$). Electroencephalograms were also recorded from brains of adult, nonmigratory rainbow trout (*Salmo gairdneri*) and of goldfish, kept under the same conditions, for comparison with the adult salmon. In both of these nonmigratory species, spontaneous electrical activity in olfactory bulbs was lower, although that of the optic lobes was much higher than in adult spawning salmon.

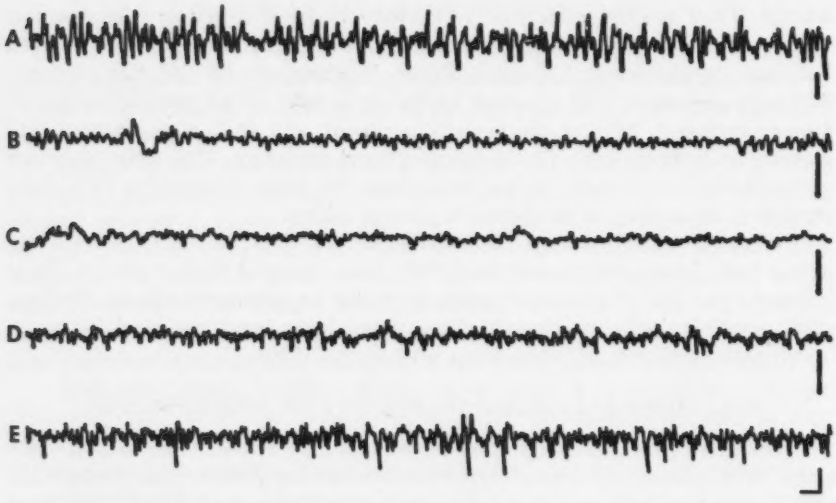

FIG. 2. EEG records from the olfactory bulb (A), cerebrum (B), optic lobe (C), and anterior (D) and posterior (E) cerebellum of a migrating adult silver salmon. Vertical calibration, 50 $\mu\bar{V}$; horizontal calibration, 1 sec (from Hara et al., 1965). (Copyright 1965 by the American Association for the Advancement of Science.)

The very low electrical activity of the optic lobes of adult salmon suggests that visual cues may be of little use during the final stages of migration. Relatively high activity in the olfactory bulb of the young salmon is consistent with the olfactory hypothesis that they are "imprinted" by an odour of the home stream (see below). However, it may be dangerous to over-stress these results obtained by recording spontaneous electrical activity of the brain before the nature of EEG is adequately explained.

SPECIFICITY OF THE RESPONSE TO HOME WATER

If there exists a specific odourous chemical or combination of chemicals in each stream, and if salmon do recognize and react to it, corresponding olfactory response will be indicated in response to "home water."

A clear stimulation in EEG patterns recorded from the olfactory bulb was obtained when home water was infused into the nasal cavity of migrating adult salmon, whereas various natural waters from other nearby sources produced virtually no response (Fig. 3; Hara et al., 1965). This response may be considered stimulatory and dependent on a factor in the home water, since it disappeared when the home water was diluted to at least 10% in distilled water (Fig. 4; and also see Oshima et al., 1969c).

These findings suggest that olfaction is important in guiding salmon during the final phases of homeward migration, and that such olfactory discrimination occurs at the level of either the olfactory bulb or the olfactory epithelium. However, there remains the possibility that certain nonspecific

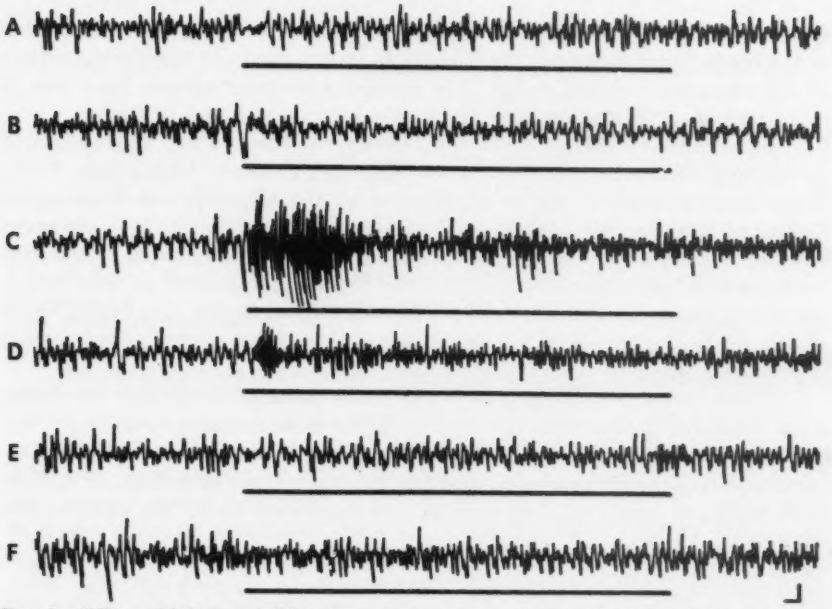

FIG. 3. Effects of infusion of different waters into the nasal sac upon EEG patterns in the olfactory bulb of a spawning salmon. A, distilled water; B, tap water; C, water from the home pond; D, water from the trout hatchery in Seward Park; E, water from Green Lake; F, water from Lake Sammamish. The lines below each record indicate duration of stimuli. Vertical calibration, 50 $\mu\bar{V}$; horizontal calibration, 1 sec (from Hara et al., 1965). (Copyright 1965 by the American Association for the Advancement of Science.)

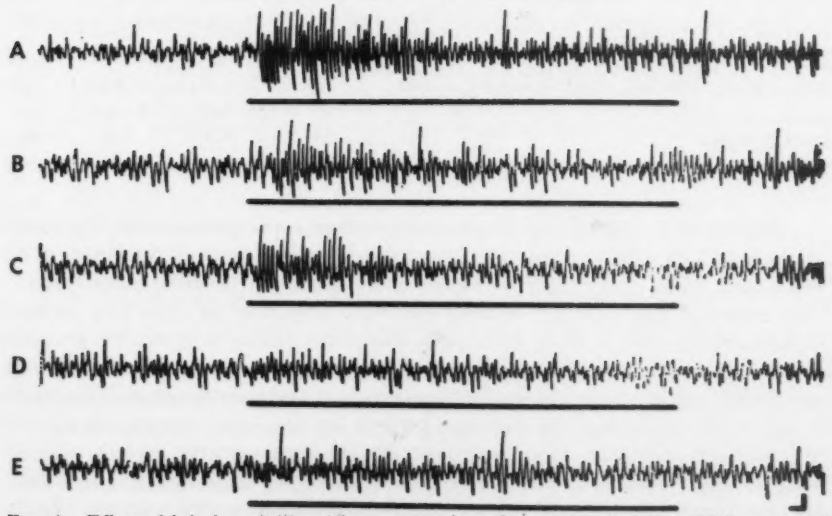

FIG. 4. Effect of infusion of diluted home water into the nasal cavity upon EEG patterns in the olfactory bulb of an adult migrating salmon. A, 100%; B, 50%; C, 25%; D, 15%; and E, 10% home water, respectively (from Ueda et al., 1967).

odourous substance(s), such as foods, were merely more highly concentrated in the home water than in any of the other waters used. To test the specificity of the response to home water, it is necessary to treat salmon from two or more termini of migration with their own home water and others'.

Such apparent specificity of the response to home water was confirmed by treating salmon from three different spawning grounds (Ueda et al., 1967). Those grounds were (i) the pond adjacent to the University of Washington College of Fisheries; (ii) a salmon hatchery operated by the State of Washington located on Issaquah Creek, which empties into Lake Sammamish; and (iii) a salmon hatchery operated by the State of Washington located on Soos Creek, which empties into the Green River. The Green River drains into Puget Sound at Elliot Bay (Fig. 1). Such a unique geological location offered an interesting experimental design.

Characteristic responses were consistently induced in all the fish tested when infused with their own home water. Little or no response could be evoked by water from spawning sites of other groups of breeding salmon (Table 2). This implies that each spawning area has its own specific stimulant, or specific combination of stimulants, recognized and responded to by the anadromous salmon.

TABLE 2. Specificity of response to home water in salmon from three spawning systems (from Ueda et al., 1967). The magnitude of response is represented as a percentage of that to home water (mean ± SE %), number of fish in parentheses.

Fish from	Water from: Univ. Wash. Fisheries pond		Issaquah Cr.		Soos Cr.	
Univ. Wash. Fisheries pond (chinook)	100	(4)	0	(4)	2.3±3.1	(4)
Issaquah Cr. (coho)	39.1±14.5	(3)	100	(3)	6.4±3.5	(3)
Soos Cr. (coho)	0	(4)	0	(4)	100	(4)

Oshima et al. (1969b) continued further these experiments under improved experimental conditions, and confirmed the basic conclusions by Hara et al. (1965) and Ueda et al. (1967), though some quantitative differences existed. They recorded the olfactory bulbar electrical responses of spawning salmon (*O. tshawytscha* and *O. kisutch*) homed into three different spawning grounds (University of Washington College of Fisheries pond, Issaquah Creek, and Soos Creek), when various natural waters were infused into the nostril. Generally, the most vigourous response was evoked by the home stream water and relatively less vigourous responses were evoked by nonnative waters with an exception that Issaquah Creek water evoked olfactory responses in University and Soos Creek salmon indistinguishable from the home water response (Table 3).

TABLE 3. Specificity of the response to home water in adult spawning salmon (from Oshima et al., 1969b).

Test water	Relative response[a]					
	Univ. Wash. Fisheries pond chinook		Soos Cr. coho		Issaquah Cr. coho	
Home water	100.0	(7)	100.0	(7)	100.0	(8)
Soos Cr.	48.8±8.5	(13)	–		63.1±10.4	(4)
Issaquah R.	101.6±9.3	(12)	99.0±10.5	(10)	–	
Skykomish Cr.	66.3±8.1	(8)	73.1±6.3	(12)	–	
Univ. Wash. Fisheries pond	–		–		74.5±6.5	(6)

[a]Values are the mean ratios (×100) of the magnitude of the home water response to the magnitudes of the nonhome water responses. The numbers of fish are in parentheses.

However, these electrophysiological findings raise several questions. The first question is how it is possible for the characteristic local smell of such a small spawning ground to be recognized at junctions or at the mouth of rivers, since, as mentioned already, the specific EEG response to the home water disappeared when it was diluted to 10% in distilled water (Fig. 4). Obviously, this does not necessarily imply that olfactory acuity to home water is of a low order. It does indicate that the recording method, although useful in demonstrating olfactory discrimination, is of only limited significance in the quantitative sense. At a stream junction it is necessary for homing salmon to have the ability to select one stream and to reject the other. It was observed that the magnitude of the response to nonnative water is invariably smaller than to the native water (Table 4; Ueda et al., 1967). Although it was suggested

TABLE 4. Discrimination of native water from nearby nonnative water where the two are alternatives (from Ueda et al., 1967). The magnitude of response to nonnative water is represented as a percentage of that to native water (mean ± SE %), number of fish in parentheses.

Fish from	Magnitude of response	
Univ. Wash. Fisheries pond (chinook)	40.1±5.2	(3)
Issaquah Cr. (coho)	49.2±8.6	(5)
Soos Cr. (coho)	24.7±6.9	(3)

by Ueda et al. (1967) that a salmon, when presented with a choice between two streams, invariably chooses and ascends the one that produces a stronger bulbar response, Oshima et al. (1969c), from their extensive studies, did not obtain a correlation between the relative intensity of the bulbar EEG response and actual behavioural performance. This situation obviously requires further study, particularly of the neural coding mechanism in both the receptor and central nervous system.

It was further observed that the salmon responded to water taken from places along their migratory routes below the spawning site, e.g., the salmon that had returned to Issaquah responded to water collected from the inlet to the University of Washington Fisheries pond, and similarly the salmon returned to the Fisheries pond responded to water collected from the Government locks (Fig. 5). Recently, Oshima et al. (1969c) have obtained more extensive data

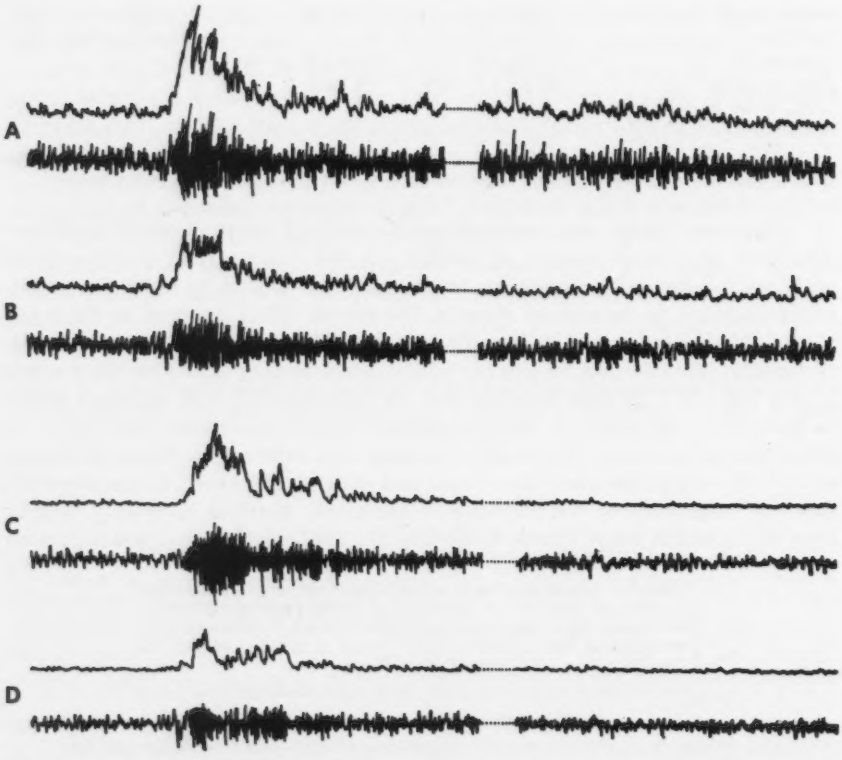

FIG. 5. Olfactory bulbar responses of University salmon (A and B) and Soos Creek salmon (C and D) to waters taken from places along the migratory routes below the spawning sites. A and D, University of Washington Fisheries pond water; B, water just above the locks; C, Soos Creek water. Upper channel, integrated EEG; lower channel, regular EEG traces (from Ueda et al., 1967).

showing responsiveness to waters along the freshwater migratory routes in three spawning ground systems mentioned above. Experiments carried on with salmon homed to the Issaquah are summarized in Table 5 (see also Fig. 1). Although largest responses were always evoked by home water for each of the three spawning groups, the responses to various waters along the migratory route were generally equivalent at least quantitatively.

TABLE 5. Olfactory response of Issaquah Creek coho salmon to waters traversed in the homing migration (from Oshima et al., 1969c).

Test water	No. fish	% of home water response (mean ± SE)
2[a] Home water	8	100.0
Soos Cr.	4	63.1±10.4
1 Univ. Wash. Fisheries pond	6	74.5±6.5
A[a] Ship canal	8	76.1±10.0
B Wayne Slough (Sammamish R.)	8	74.7±11.6
C L. Sammamish	8	55.6±9.2
D Issaquah R.-L.	3	93.6±12.1
E East Fork, Issaquah Cr.	8	63.4± 4.8
F Issaquah Cr.	8	90.5± 7.7

[a]Figures and letters indicate sites of collection of water samples used as shown in Fig. 1.

It seems unlikely that such bulbar activity was evoked by diluted home water, since the electrical response to home water disappeared when it was diluted at least 10-fold with distilled water (Fig. 4). It is then suggested that these responses were evoked sequentially by factors characteristic of waters traversed on the way to the spawning site. It seems that on returning from the sea, the adult salmon retrace a trail of stimuli in a sequence that is the reverse of that *imprinted* in the young fish on their seaward migration. A similar sequential hypothesis for home stream detection has recently been described by Harden Jones (1968). The application of a technique that could show a qualitative difference between agents acting on the olfactory receptor would greatly help to solve the problem.

Even if specific EEG patterns could be induced in the olfactory bulb in response to applications of the home water, this does not immediately imply that the salmon has distinguished the home stream water from others. Salmon might simply show preference or fright reaction to it. For a salmon to return precisely to its home stream, responses to water must be differential, not simple response such as that to a repellent or attractant. Finally, the nature of the olfactory bulbar response after stimulation of the olfactory receptor with home water, or even the nature of EEG itself, is still unknown. At present there seems to be no satisfactory explanation for this.

In all the experiments in which olfactory bulbar responses to various natural waters were recorded, the stimulus was always given after the nostril was rinsed with distilled water. Therefore, responses evoked in the olfactory bulb represented the activity change from this level. Such an experimental situation appears quite different from those of natural conditions in river systems. Obviously, environment surrounding the olfactory epithelium before stimulation is very important in olfactory recognition, and the responses thus evoked are easily affected by various factors in the course of preparation and recording.

NATURE OF THE CHEMICAL CLUE

An attempt has been made by scientists of the Fisheries Research Board of Canada, Vancouver Laboratory, to isolate and identify the home stream odour substance by observing the behaviour of the fish. Idler et al. (1961) captured sockeye salmon on their spawning migration to Great Central and Cultus lakes, Vancouver Island, British Columbia; they examined the responses of the fish to various waters by recording activity changes, such as dispersion of schools or increase in swimming speed, with a cine camera. The fish from both lakes responded positively to their own home stream water but not to other waters. When the home water was lyophilized at low temperature, the fish responded to the volatile fraction but did not respond to the residue. The fish did not respond to either the distillate or to the residue when the water was distilled under reduced pressure at about 70 C. After the lake water was autoclaved at 100 C for 3 hr the fish no longer responded to it. The effluent obtained by passing the lake water through a mixed bed of strong base and strong acid ion-exchange resins produced a positive response. Furthermore, the fish did not respond to the nondialyzable material after the lake water was dialyzed for 72 hr against running tap water at 8–10 C. These results indicate that the unknown active component(s) is volatile, water-soluble, neutral, dialyzable, and heat-labile, and agree with the findings of Hasler and Wisby (1951) with the bluntnose minnow (*Hyborhynchus notatus*).

Using adult spawning sockeye salmon, Fagerlund et al. (1963) confirmed the previous results of Idler et al. (1961), and further demonstrated that the recombined volatile and nonvolatile fractions of the home stream water induced a stronger response than did the volatile fraction alone. This finding suggests that a nonvolatile material may play some part in stimulating the fish.

McBride et al. (1964) trained sockeye salmon smolts to distinguish between waters from two natural sockeye nursery systems. They showed that the stimulatory material for smolts from each water was also volatile, and that the volatile material collected from the lake water could evoke a response in the group of fish previously conditioned to it.

In spite of these efforts the kind of chemicals that give the home stream its characteristic odour is still unknown. As suggested by Wright (1964), the home stream odour that guides the salmon may be the same as the home range odour of one or other of the local, nonmigratory fish species. In connection with this, it is noted that salmon returned to the University of Washington College of Fisheries pond showed a visible response to the water taken from a pond of a trout hatchery on the shore of Lake Washington (Fig. 3D).

Oshima et al. (1969b) reported that the chinook salmon, reared in fresh water for 2 years, showed higher olfactory bulbar response to the water in which a few chinook salmon had been kept overnight. Keeping coho salmon under the same condition did not make the water stimulatory.

MECHANISM OF LEARNING

As already mentioned, salmon do not return simply to the stream their parents returned to, but to the stream in which they themselves were hatched and spent their young period. Then, how do young fish learn the characteristics of their home stream water? This phenomenon has long been discussed in terms of imprinting (see Hasler, 1966; Harden Jones, 1968). Imprinting is a rapid learning process restricted to a certain brief period of life usually at an early stage in development. It has been studied primarily in ducklings and chicks, and it is still uncertain whether or not such a sharply defined critical or sensitive period exists (Bateson, 1966). In fish nothing is known concerning the time of the sensitive periods, or the duration of the imprinting process. No systematic study has been carried out with particular emphasis on this problem. The only data available are those obtained from transplantation experiments by Donaldson and Allen (1957) and by the International Pacific Salmon Fisheries Commission (1953).

Donaldson and Allen (1957) transplanted coho salmon fingerlings, hatched in early 1951 and reared until January 1952 at Soos Creek hatchery, to both the Issaquah hatchery and the University of Washington College of Fisheries hatchery. The fingerlings were marked and released in March 1952. These coho salmon returned, when adult, to the releasing points in the autumn and winter of 1953–1954. This indicates that imprinting occurred between January and March during their 2nd year of freshwater life. Evidence from recent transplantation experiments with Atlantic salmon has led Carlin (cited from Hasler, 1966) to believe that only a few weeks of exposure to a natural water are necessary for imprinting, and that imprinting can occur when the smolts are as long as 20 cm and in their 2nd year of hatchery life.

Shirahata and Tanaka (1969) reported some results of their transplantation experiments with a landlocked sockeye salmon, *O. nerka*, in Lake Chuzenji in central Japan. In May 1966 they transplanted 183,000 fingerlings that had been reared for 6 months after hatching in a pond at the Freshwater Fisheries Research Laboratory to a creek where no salmon had previously been taken. In fall of 1968, 1271 returned as adults to the creek. They believe that the time necessary for the fish to be imprinted to the home stream is less than 10 days, because the fish transplanted were observed to go to the lake in that time.

Thus, "the young salmon may undergo a series of imprinting processes corresponding to each major change of environment made in freshwater: gravel bed, lake outlet, tributary river, main river. It could be important that the sequence in which these imprints are made should correspond exactly to the reverse sequence of stimuli that the upstream migrant receives on the way home" (Harden Jones, 1968).

Although all these observations do not define the sensitive period or the duration of the imprinting process, they do seem to indicate a consistent type of homing mechanism. Future investigations of salmon migration should be focused on this particular problem not only to test the hypothesis but also

to contribute to a problem of protecting salmon runs from water pollution or other man-made changes. It will probably take a long time to conduct the diverse field tests, to prove the validity of the mechanism of the imprinting. Electrophysiological techniques have shown their usefulness in the study of home stream discrimination in salmon. It also seems promising that the imprinting process will be successively monitored by recording development of the olfactory response to odourous factors in a newly introduced environment.

Oshima et al. (1969b) observed alteration in olfactory bulbar responses of migrating chinook salmon to Soos Creek water and to University of Washington Fisheries pond water after transporting the fish from the Soos Creek to the University Fisheries pond. They concluded that olfactory responses of even spawning adults increased from an initial low value until about 3 days after transfer to a new environment.

Recent reports indicate that sensory stimulation elicits changes in brain RNA and protein. There also seems to be agreement that RNA synthesis is part of a memory-establishing mechanism. Rappaport and Daginawala (1968) studied the changes in the synthesis of brain nuclear RNA during olfactory stimulation in marine catfish, *Galeichthys felis*. They showed that olfactory stimulation with morpholine induced an increase in brain nuclear RNA and a change in base ratios in both intact and split-brain preparations in isolated heads of catfish. Oshima et al. (1969a) recently reported that intracranial injection of puromycin, actinomycin D, or cycloheximide in the homing chinook salmon markedly inhibited olfactory bulbar discrimination between home water and other natural waters, including that from spawning sites of other groups of salmon. The blockage was marked a short time (4–7 hr) after administration, and at longer intervals (9–28 hr) there was a partial restoration of olfactory memory-based discrimination. From these data they concluded that long-term olfactory memory in homing salmon depends upon continued metabolism of RNA and continued protein synthesis.

If, furthermore, learned behaviour is transferred through preparations of brain RNA (Reinis, 1968), which means persistence of long-lived RNA or protein, or both, will a salmon returning to its home stream respond to waters from the spawning sites of other groups of salmon after its brain has been injected with RNA preparations of the latter? This critical experiment has not yet been performed.

Sublethal concentrations of pesticides, e.g., DDT, act on either peripheral or central (or both) nervous structures of fish (Anderson, 1968; Aubin and Johansen, 1968; Anderson and Peterson, 1969). If DDT and other related pesticides owe their activity to the formation of a charge-transfer complex with a nervous component as suggested by O'Brien and Matsumura (1964), it would be likely that the imprinting process of the young fish will be seriously affected when exposed to them. Electrophysiological as well as biochemical studies of the imprinting process at a macromolecular level are to be strongly encouraged.

ACUITY OF THE OLFACTORY SENSE OF FISHES

A temporary reduction in the rate of upstream migration of adult spawning salmon occurs when human hands are rinsed in the water above the fish; even spitting in the salmon ladder will cause a brief reaction (Brett and MacKinnon, 1954; Alderdice et al., 1954). The water rinses of many mammalian skins, e.g., those of bears and sea lions, have the same effect. The active substance, L-serine, was isolated from extracts of mammalian skin by Idler et al. (1956, 1961). Since this substance can elicit a reaction at the extremely high dilution of 1.3×10^{-10}, the olfactory sense was suggested (Brett and MacKinnon, 1954). This is clearly a type of alarm reaction, and is believed to play an important role in protecting the salmon from various prey during the spawning migration.

The phenomenon just described is only one example of the very highly developed sense of smell in fish; other studies may be briefly reviewed. Neurath (1949) trained minnows (*Phoxinus phoxinus*) to discriminate β-phenylethyl alcohol and eugenol, and determined the threshold values 4.3×10^{-10} and 6.0×10^{-11}, respectively. The rainbow trout, *Salmo irideus*, detected β-phenylethyl alcohol at dilutions of 1×10^{-9}; this is nearly the same order of sensitivity as in humans (Teichmann, 1962). Teichmann also trained young European eels, *Anguilla anguilla*, to detect β-phenylethyl alcohol, *l*-menthol, citral, eugenol, and ionon. He established the thresholds for each; they ranged from dilutions of 3.5×10^{-19} (β-phenylethyl alcohol) to 2×10^{-16} (ionon). The eel is thus in the same class as the dog in its olfactory sensitivity. Roaches, *Leuciscus rutilus*, responded to benzol at dilutions of 2.0×10^{-7} M and to phenol at 9.5×10^{-6} M (Marcström, 1959). According to Teichmann's estimation, the presence of only a few molecules of such chemicals in the olfactory sac is required to evoke the smell sensation.

Recently, attempts have been made to investigate the olfactory function of fishes using electrophysiological techniques. Generally, however, olfactory thresholds using electrophysiological changes as indices are higher than those determined in behavioural tests. Boudreau (1962) recorded summated electrical activity from the olfactory tract of the catfish *Ictalurus catus* and *I. melas*; he demonstrated that the increase in tract activity was produced by introducing various chemicals into the olfactory sac in extremely dilute concentrations. Acetic acid and butyric alcohol remained effective at concentrations of 10^{-11} M and 10^{-15} M, respectively; these figures are comparable to those obtained from the behavioural studies mentioned earlier.

In some teleosts the olfactory tract runs as a long nerve bundle between the bulb and telencephalon (cf. Hara, 1970). This unique anatomical feature of the olfactory system provides a convenient site for the electrophysiological analysis of messages conveyed through the olfactory tract to higher centres. Nanba et al. (1966) recorded the spontaneous firing of single fibres in the olfactory tract of *Abramis abrama* and *C. auratus*; it was demonstrated that a fibre exhibits different patterns of activity when stimulated with different

odours and that different fibres respond to the same odour in different ways. This indicates the existence of olfactory discrimination at the receptor level. Similar discrimination in the olfactory system of other teleost fish has been reported (Döving, 1966; Hara, 1967a, b). Döving (1966) recorded the nervous activity of single secondary neurones from the olfactory tract of the burbot (*Lota lota*) while stimulating the olfactory epithelium with various chemical solutions. About 30% of the stimuli produced an increased activity in the neurones, and 20% caused inhibition of the spontaneous discharge; about half of the stimulations failed to produce any change in the firing rate. Hara (1967a) also recorded spontaneously firing action potentials from the neurones in the olfactory bulb of the goldfish by using microelectrodes. Individual neurones responded differently to chemical stimulation of the olfactory epithelium. Spontaneous activity of more than 60% of the bulbar secondary neurones tested was either facilitated or inhibited when stimuli were given.

Earlier, Shibuya (1960) recorded resting potentials of the olfactory epithelium in some teleosts using glass microelectrodes. Potentials were 12.4 mV in *Anguilla*, 8.7 mV in *Misgurnus*, 8.6 mV in *Parasilurus*, and 7.6 mV in *Channa*, *Cyprinus*, and *Entosphenus*. Slow negative potentials were induced in the mucosa when stimulated with odourous fluids such as butyric acid or extract of silkworm pupae (Fig. 6). The shapes of the potentials were species-

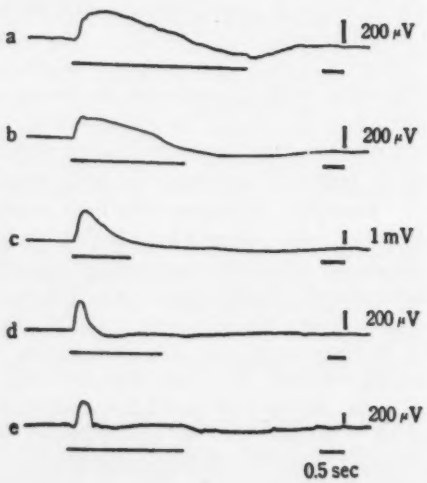

FIG. 6. Slow potential induced in the olfactory mucosa when stimulated with extract of silkworm pupae. *a*, *Channa argus*; *b*, carp; *c*, lamprey; *d*, catfish; *e*, eel (from Shibuya, 1960).

specific. In *Parasilurus* and *Anguilla* it had a short duration (0.4–0.7 sec) with a rapid decline. In the eel it always had a duration of about 0.4 sec regardless of stimulus duration (Fig. 6). However, there has been much argument concerning the nature of the slow mucosal response (Ottoson, 1963; Moulton and Tucker, 1964). Although an attempt has been made to relate olfactory sensitivity to the short duration of the mucosal potentials, more precise analyses

of the receptor response are required by recording activity in single receptor cells.

There appears to be a relationship between nasal and genital functions, whereby sex hormones influence the function of the olfactory organ. Hara (1967b) studied the effect of estradiol and testosterone on evoked potentials in the olfactory bulb of the goldfish after chemical or electrical stimulation of the olfactory epithelium. Administration of estradiol markedly increased these olfactory bulb responses (Fig. 7). The effect of the hormone was much greater in males than in females. From further analyses, Hara (1967b) suggested the existence of a hormone-sensitive centre in the diencephalon or telencephalon, and of a feedback system that regulates the function of the olfactory bulb through centrifugal fibre systems.

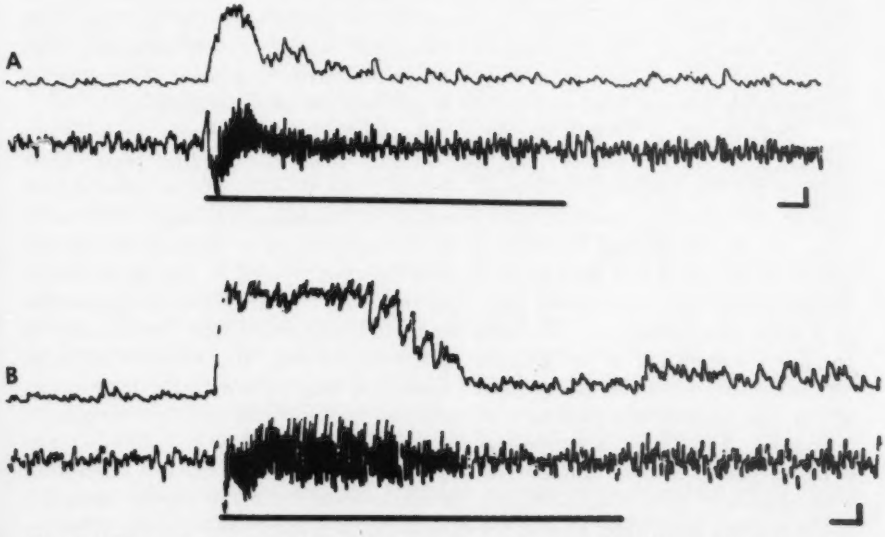

FIG. 7. Effect of estradiol on the electrical responses induced in the olfactory bulb of goldfish after infusion of NaCl solution into the nasal cavity. A, control; B, estradiol-treated (25 μg/fish per day for 10 days) (from Hara, 1967b).

The plasma concentrations of the steroid hormones of salmon show seasonal fluctuations coinciding with various physiological states during their life cycle (e.g., Cedard et al., 1961), and enhanced secretion of gonadal steroids has sometimes been implicated as a triggering factor in spawning migration. However, nothing is known concerning a possible role of gonadal hormones in increasing the sensitivity of the nervous system of migrating salmon. The olfactory threshold of the European eel (*A. anguilla*) also showed seasonal fluctuations; it was lowest in late winter and in midsummer, and about 60 times higher in late fall and in early winter (Teichmann, 1962).

MECHANISM OF ORIENTATION

To guide salmon to their home, the stimulus received by them must be directive, that is, the concentration gradient of the odourous substance must be steep enough to elicit a klino- or tropo-taxis. Brett and Groot (1963) described the existence of considerable discontinuities in a large stream where a small stream discharges masses of turbid water. Gradients steep enough to elicit taxes may occur on the boundaries between such water masses or close to the final localization of an odour. With the exception of the latter instance there is no directive component to the odour source at all; salmon may be able to move into the water mass containing the home stream odour, but they will not obtain any further information as to the direction in which to swim. Thus, as stated by Harden Jones (1968) a home stream odour would be "more effective as a simple *sign stimulus* which releases a pattern of behaviour, for example a positive rheotropic response, which would take the fish upstream towards the home." In this case, of course, fish must have the necessary clues to orientate to the water flow. In connection with this view the following experiments carried out with chinook salmon are very interesting.

DeLacy et al. (1969) displaced spawning chinook salmon to locations in the watershed upstream and downstream from the holding pond. Over 60% of the fish displaced in several parts in Lake Washington returned downstream to the holding pond at the University of Washington College of Fisheries (Fig. 1). By comparing the differences in percentages of returns and in the delays involved in the returns of the fish between upstream and downstream displacement, they concluded that olfaction was not involved in recognition of a route downstream to the home pond. It is suggested that the absence of the home stream cue or the presence of a foreign odour may evoke a negative rheotaxis, and thus the fish displaced upstream may return to the location at which the appropriate olfactory stimulation again elicits positive rheotactic behaviour. This view is supported by further experiments in which returns of fish displaced to Lake Sammamish, 15 miles upstream from Lake Washington, were observed. More than 3 weeks after displacement, 12% of the fish returned to the holding pond after having traveled a complex route downstream (Fig. 1). In addition, it should be noted that some of the fish with the nose occluded were captured in the saltwater gillnet several miles out in Puget Sound. Thus it appears evident that the salmon tend to move downstream in absence of home stream odour.

Creutzberg (1961) has shown that elvers of the eel *A. anguilla* probably migrate from the sea to fresh water by using tidal streams. He studied the rheotropic response of elvers in an experimental chamber, and demonstrated that on ebb tides the elvers swam against the current and at high velocities burrowed into the bottom, whereas on flood tides they rose from the bottom and either swam with or were carried passively by the current. He also showed that the behavioural change of the elvers to the current was associated with an unidentified chemical substance of inland water. Recently, Miles (1968) reported that elvers of the American eel, *Anguilla rostrata*, show a stronger

positive rheotaxis to fresh water than to salt water. The attractiveness of the fresh water was shown to be due to dissolved and particulate organic matter, which was bio-degradable, heat-stable, and nonvolatile. The presence of adult eels in the water made it more attractive, whereas the presence of elvers made it less attractive.

Kleerekoper (1967a, b) analyzed the mechanism of orientation through olfaction in some marine and freshwater fishes. Single specimens were placed in an experimental tank, which was divided into 16 partial compartments by radially placed walls. The direction of the fishes' movement, left- and right-hand turns, speed of locomotion, the frequency of entries into any one compartment, the frequency of pathways, and the diurnal distribution of total activity were continuously and automatically recorded with or without odourous stimulation. None of the fishes studied moved randomly under experimental conditions devoid of directional cues. The radius of the curve in changing direction was fixed within relatively narrow limits and seemed to be species-specific; left- and right-hand turns were not evenly distributed. Such a locomotor behaviour resulted in a nonrandom pattern of relatively rigid parameters. Introduction of an odour without directional cues caused a drastic change in these parameters; the radius of the curve in changing direction decreased and the ratio of left-handed to right-handed turns was greatly changed. When an attractant odour was introduced unidirectionally, none of the species studied could locate the source, unless the odour was associated with a differential rate of water flow. It is suggested that an attractant odour releases rheotactic response, so that the localization of the source of the odour takes place through rheotaxis rather than through osmotropotaxis. Furthermore, he proposed that this mechanism forms the basis for homing orientation in those migrating species in which olfactory cues are required for the localization of spawning grounds in river systems.

Continuous excitation of the olfactory organ induces sensory adaptation or fatigue so that the given odour is no longer perceived. A home stream odour, if it is as a direct or sign stimulus, would be considered to stimulate the olfactory receptor organ continuously along the migrating route of salmon. Therefore, it is likely that the behaviour of a salmon ascending the river system is analagous to that of a dog following an odour track. The dog does not stay exactly on the track but progresses along it by crisscrossing, responding to the presence and absence of the scent as it follows its prey (Hasler, 1960). In this way salmon can overcome the adaptation and ascend the river to reach spawning grounds.

There occurred an immediate adaptation in the olfactory bulbar response in salmon after the initial activity caused by infusing the home water (Fig. 3). The mechanism of such an adaptation as detected by electrical response has recently been analyzed in the teleosts. Nanba et al. (1966), recording the spontaneous firing of single olfactory tract fibres, observed that mechanical stimulation such as water flow rapidly affected the olfactory response, so that during mechanical stimulation of the olfactory mucosa, olfactory adap-

tation to an odour was suppressed. The creation of turbulence as the fish swings its head may correspond to the "sniffing" of mammals and help the fish to overcome adaptation and to localize the source of the odour.

The olfactory bulb and other central nervous structures are probably more important than the receptor in determining sensory adaptation. In the goldfish, section of the ipsilateral olfactory tract caused marked changes in the induced responses of the olfactory bulb to chemical or electrical stimuli of the olfactory mucosa (Hara and Gorbman, 1967; Fig. 8). These results

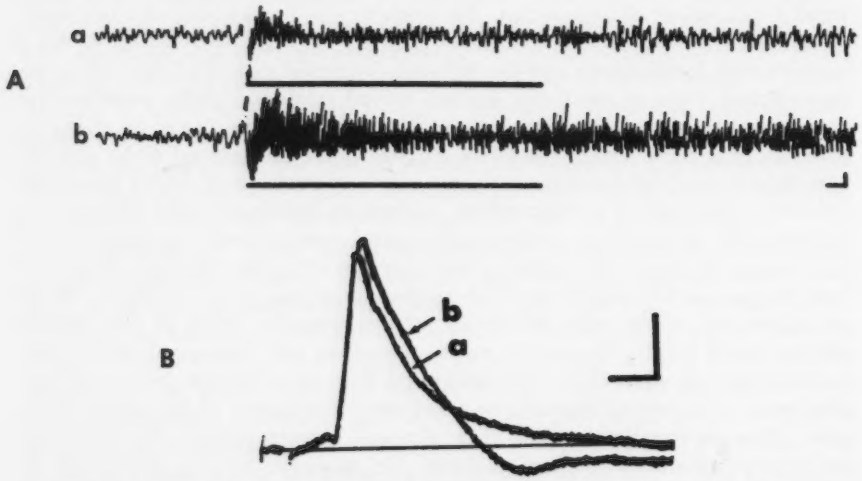

FIG. 8. Modification of the olfactory bulbar responses induced by infusion of NaCl solution into the nasal cavity (A) and by electrical stimulation of the olfactory mucosa (B) after sectioning the olfactory tract. *a*, before tract section; *b*, after tract section; *a* and *b* reversed in B (from Hara and Gorbman, 1967).

verify that the rapid adaptation seen in the olfactory bulb of the intact animal is of central origin. Furthermore, electrical stimuli applied to the opposite bulb or to the anterior commissure, or strong chemical stimuli given to the opposite nostril, depressed both intrinsic and afferent-induced electrical activity of the bulb. These findings indicate that olfactory bulbar responses to afferent stimuli can be modulated by influences from the other bulb and from more posterior parts of the brain.

Particularly in fishes, such a mechanism of central regulation, including interbulbar connections, may play an important role in the orientation towards or away from a stimulus source by simultaneous bilateral equating of stimulation.

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NOTES

Atresia and Year-Class Abundance of Northern Pike, *Esox lucius*, in Two Missouri River Impoundments

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JUNE, F. C. Atresia and year-class abundance of northern pike, *Esox lucius*, in two Missouri River impoundments. J. Fish. Res. Bd. Canada 27: 587–591.

Widespread atresia in northern pike ovaries was associated with low year-class abundance in three successive years, 1966–68, in Lake Oahe and Lake Sharpe, South Dakota, two large Missouri River impoundments. Atresia was associated with fluctuations in water temperature and level that apparently interrupted spawning.

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IN THE COURSE OF STUDYING the reproductive cycle of northern pike (*Esox lucius*) in Lake Oahe and Lake Sharpe, two large Missouri River impoundments in South Dakota (1.52×10^5 ha and 2.26×10^4 ha, respectively), I encountered an uncommon number of ovaries with numerous atretic mature ova during spawning in 1966. The high incidence of atresia recurred in 1967 and 1968 and suggested to me that it was a major cause of low year-class abundance in three consecutive years. This note describes the condition in northern pike, considers its relation to apparent year-class abundance, and discusses its possible causes.

Materials and methods — Ovaries of 630 sexually mature northern pike collected in three spawning localities in Lake Oahe and two in Lake Sharpe provided the basic materials for study. Fish were caught during the spawning period (late March to early June) with 1.2×1.8-m trap nets (19-mm bar mesh) and a 106.7×1.8-m gillnet consisting of seven 15.2-m panels of different mesh sizes (19- to 89-mm bar mesh). A thermograph furnished a record of water temperature in each locality.

Ovaries were excised, classified as maturing, running ripe, or spent, and preserved in 5% formalin. A sample of ova was removed from each pair of maturing and running-ripe ovaries, placed in a Sedgewick-Rafter cell, and examined microscopically. If atretic late-maturing or mature ova appeared in the sample, sufficient numbers were added to fill the cell one layer deep and counts were made of atretic and normal ova in the sample. Total counts for individual samples ranged from 130 to 227 ova and averaged 175. Residual maturing or mature ova found in spent ovaries were also examined for evidence of prespawning atresia.

Most atretic late-maturing and mature ova were distinguished by the following criteria. They were semitransparent to transparent (normal ova are translucent), pale yellow to colorless, and often with irregularly shaped vacuolated areas in the yolk mass. In early stages of atresia the yolk mass was usually diminished in size, thus creating a transparent zone of varying width

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between the chorion and the yolk. The oil globules were pale yellow to colorless or absent. In advanced stages of atresia the chorion was collapsed and the yolk reduced to an amorphous mass or scattered in small blobs and filaments. Atretic mature ova were slightly adhesive to nonadhesive, whereas normal mature ova were strongly adhesive. Normal and atretic ova of northern pike are shown in Fig. 1.

Since atretic ova were in varying stages of degeneration, it was impossible to decide if some ova were normal or atretic. Greatest difficulty was experienced in recognizing incipient stages of atresia. Arbitrarily, ovum samples with 10% or more atretic late-maturing or mature ova were designated as "atretic." Those in which the ova could not be classified with confidence and those in which 2–9% of the ova were atretic were designated as "questionable." Samples with 1% or fewer atretic ova were designated as "normal."

To test the fertility of some ova classified as questionable, sperm from a single male was added to four lots of mature normal ova and four classified as questionable and the success of fertilization determined. The ova came from eight running-ripe females taken in a single net catch in 1967. After water-hardening the ova were transferred to glass jars, approximately 100–150 ova per jar, covered with plastic screen, and placed on the bottom in the spawning locality where the females had been captured. After 4 days of incubation the jars were removed from the water and the dead ova and living embryos in each jar counted.

Measures of apparent year-class abundance of northern pike at age 0 were made in Lake Oahe from 1965 to 1968 and in Lake Sharpe beginning in 1966. Fishing was conducted from mid-June to mid-September with a 30.5 × 2.4-m haul seine (6-mm bar mesh) in the littoral zone and an 8.0-m otter trawl (19-mm bar mesh codend and 6-mm bar mesh liner) in deeper waters. Periodic sampling with a graduated-mesh gillnet, described earlier, furnished an additional measure of year-class abundance at age 1.

Ancillary information on the fertility of northern pike ova in 1965–68 was extracted from hatchery production records of the South Dakota Department of Game, Fish and Parks and the Bureau of Sport Fisheries and Wildlife. Ova from running-ripe females caught in several localities in Lake Oahe and Lake Sharpe were manually fertilized in the field and incubated at the Pickerel Lake State Fish Hatchery, Waubay, S. Dak. In 1968, manually-fertilized ova obtained from the Lake Sharpe pike population were also incubated at the Gavins Point National Fish Hatchery, Yankton, S. Dak.

Incidence of atresia — Atresia was observed most frequently in mature ova of running-ripe ovaries. At least half of the mature ova in 32–63% of the samples from running-ripe ovaries examined in any 1 year were atretic, and all mature ova in some samples were atretic (Table 1).

TABLE 1. Numbers and percentages of samples from running-ripe northern pike ovaries with various percentages of mature atretic ova, 1966–68.

% frequency of atretic ova	1966		1967		1968	
	No. ovaries	%	No. ovaries	%	No. ovaries	%
0–1	2	5	1	2	15	13
2–9	6	16	11	17	11	9
10–24	13	35	13	20	9	8
25–49	4	11	5	8	8	7
50–99	10	27	23	36	21	18
100	2	5	11	17	53	45

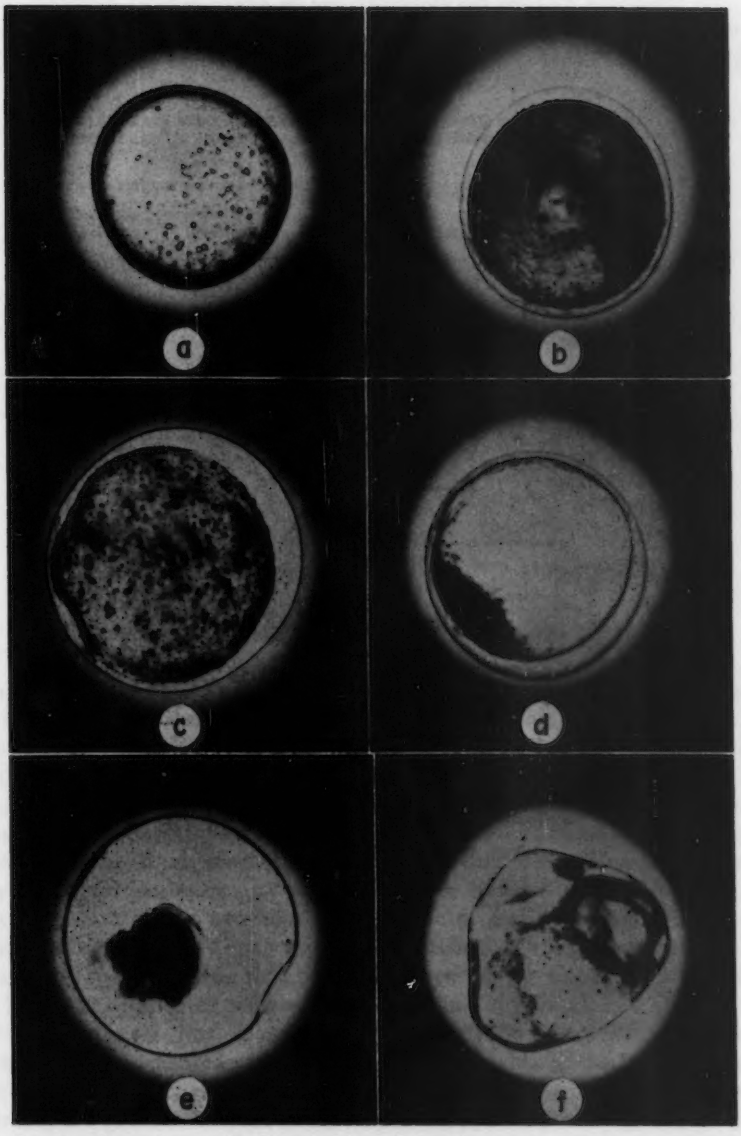

FIG. 1. Photomicrographs of ovulated northern pike ova taken from running-ripe ovaries. ×10. (*a*) Normal mature ovum. (*b*) Mature ovum in early stage of atresia; yolk mass has diminished in size, and several vacuolated areas have appeared (dark areas). (*c*) Later stage of atresia showing the beginning of disintegration of cell contents; at this stage the ovum is semitransparent. (*d*) Advanced atretic ovum in which the yolk and oil droplets have become reduced; the cell membrane is still intact. (*e*) Transparent late-stage atretic ovum in which all cell contents form an amorphous mass. (*f*) Collapsed chorion of a late-stage atretic ovum.

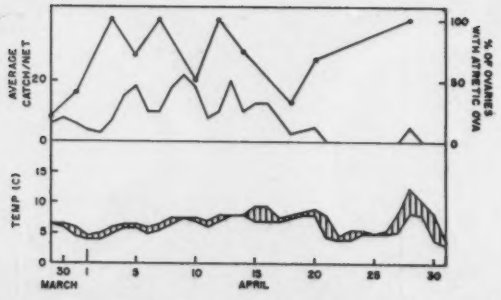

FIG. 2. Comparison of the catches of running-ripe northern pike females (solid line), the percentages of running-ripe ovaries classified as atretic in collections (solid dots and line), and the daily minimum and maximum water temperatures (hatched lines) at the upstream sampling location in Lake Oahe, 1967.

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Approximately three-fourths of the running-ripe ovaries examined each year were classified as atretic and much of the remainder as questionable (Table 2). Test lots of manually-fertilized ova incubated in one of the spawning

TABLE 2. Frequency of occurrence (%) of northern pike ovaries in the three maturity stages classified according to the extent of atresia, 1966–68.

Spawning period	No. ovaries	Normal	Atretic	Questionable
		Running ripe		
1966	39	5	77	18
1967	74	5	71	24
1968	121	15	74	10
		Maturing		
1966	101	98	3	0
1967	114	97	2	1
1968	64	83	6	11
		Spent		
1966	42	12	45	43
1967	58	3	12	84
1968	17	53	41	6

areas in 1967 showed survival to be much lower for those ova classified as questionable (0–2%) than for normal ova (45–63%). This suggests that the extent of atresia within ovaries classified as questionable may have been even higher than was indicated from the microscopic examination.

Each year, a few maturing ovaries were found in which most or all ova in advanced developmental stages were atretic (Table 2). Some spent ovaries contained a few residual mature ova that apparently were either normal or in early stages of postspawning atresia. Others, however, contained many unovulated late-maturing ova that were atretic or freshly ovulated mature ova that were in advanced stages of atresia. Relatively high frequencies of such partially spent ovaries that were classified as atretic or questionable (Table 2) further indicated widespread atresia in 1966–68.

Atresia and year-class abundance — The high incidence of atresia in northern pike was associated with low apparent abundance of the 1966 through 1968 year-classes. Catches of age 0 or age 1 pike of these year-classes were considerably lower than catches of 1965 year-class fish (Table 3). Although complete ovary-catch data for 1965 are lacking, preserved ovum samples from 44 running-ripe ovaries collected in Lake Oahe in 1965 were available for comparison. Reexamination of these samples showed a lower incidence of atresia (39% contained 1% or fewer atretic ova, and 57% contained fewer than 10%) than in any of the following 3 years.

TABLE 3. Measures of apparent abundance of age 0 and age 1 northern pike of the 1965–68 year-classes in the two impoundments.

	Seine			Trawl			Gillnet		
Year-class	No. age 0 fish	No. hauls	Catch/ haul	No. age 0 fish	No. hauls	Catch/ haul	No. age 1 fish	No. sets	Catch/ set
Lake Oahe									
1965	144	208	0.69	43	62	0.69	27	19	1.42
1966	6	334	0.02	1	52	0.02	1	30	0.03
1967	4	262	0.02	0	30	0	3	27	0.11
1968	0	204	0	–	0	–	–	–	–
Lake Sharpe									
1965	–	0	–	–	0	–	30	71	0.42
1966	15	119	0.13	–	0	–	4	40	0.10
1967	0	197	0	0	44	0	0	56	0
1968	1	240	<0.01	0	60	0	–	–	–

A lower fertility of northern pike ova obtained from Lake Oahe and Lake Sharpe spawners in 1966–68 was also evident from production records of both State and Federal fish hatcheries. The average percentage hatch in 1966–68 was only about half that in 1965 (Table 4).

TABLE 4. Percentage hatch within lots of ova stripped from Lake Oahe and Lake Sharpe northern pike females, 1965–68.

Year	No. ova (*millions*)	No. lots	% hatch	
			Range	Mean
1965	16[a]	4	54–76	66
1966	23[a]	10	0–42	23
1967	17[a]	12	16–61	33
1968	10[a]	11	0–61	18
	5[b]	8	10–90	28

[a]Incubated at the Pickerel Lake State Fish Hatchery.
[b]Incubated at the Gavins Point National Fish Hatchery.

These findings suggest to me that the intraovarian mortality of ova was basically responsible for the low year-class abundance of northern pike in 1966–68.

Causes of atresia — I conclude that the high incidence of atresia in northern pike ovaries probably resulted from interruptions of spawning that were associated with fluctuations in water temperature or level. The catch of running-ripe females declined, for example, whenever the water temperature regimen

in the littoral zone showed a downward trend. In some instances the fish disappeared and did not return to a given locality even though water temperature subsequently rose to previous or higher levels. Generally, the more severe and prolonged the cold spell, the fewer the number of running-ripe females that returned to the spawning locality and the higher the incidence of atresia. Moreover, most of the running-ripe ovaries classified as atretic and questionable were encountered during the first several weeks of spawning in every year. The dates when such ovaries first appeared varied from March 21 in 1967 to March 30 in 1966, and over 50% of these ovaries were taken before mid-April in 1967 and 1968 and by the end of April in 1966. An example of the apparent relation of catch of running-ripe females, water temperature, and incidence of atresia among running-ripe ovaries is provided from the upstream location in Lake Oahe in 1967 (Fig. 2).

Sudden lowering of the water level at times resulted in reduced catches of running-ripe females or prevented the fish from entering previously used spawning areas. Such events were usually followed by an increased incidence of atresia. On April 9, 1968, for example, a reduction in water-discharge volume through Oahe Dam (1047 m^3/sec to 147 m^3/sec) over a 6-hr period resulted in a 1-m drop in water level and exposure of the bottom at the downstream sampling location in Lake Sharpe. Prior to the drop in water level, 16 out of 17 (94%) running-ripe ovaries examined from this location were classified as normal. Two days after reduction in water discharge, only 5 out of 18 (28%) running-ripe ovaries were classified as normal.

Whatever the cause of widespread atresia, its implication with low year-class abundance of northern pike suggests that the phenomenon ought to be investigated further in both freshwater and marine fish stocks.

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Estimation of Bone Material in Fish Protein Concentrates

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An improved method for estimation of bone material in fish protein concentrates prepared from cod and herring is proposed. It is based on the difference of densities of dried protein and bone material.

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THE SKELETAL DEBRIS is the main carrier of mineral salts in fish protein concentrates (FPC). The bones of marine fishes are known to be particularly rich in minerals. Only part of these minerals could be expected to be extracted during the production of FPC. The concentration of bone material in FPC prepared from whole fish and particularly from filleting scraps may exceed 30%, thus considerably decreasing the nutritional value and the textural qualities of the product. There is a definite need for a simple method that would allow us to determine rapidly the content of bone material in FPC with a reasonable degree of accuracy.

Of the various possible mechanical ways of bone separation from FPC, such as sifting, winnowing, and flotation, the flotation seemed to be most promising. The method of flotation with variable success has been previously proposed for the determination of bones and grit in animal feeds. Vahlkampf (1936) used carbon tetrachloride as the flotation liquid and found that on the average only 10% of the bones were separated. Consequently he recommended the use of a factor of 10 to calculate the final result. However, Claussen (1937) found this method unreliable. The Association of Official Agricultural Chemists' method (1965, p. 339) designed for the determination of grit and bones in coarsely ground feeds, particularly in tankage, gave erratic results when applied to the finely ground FPC, particularly when the FPC was made from herring. Hill and Hites (1968) also found that the Association of Official Agricultural Chemists' method (1965, p. 339) is not applicable to very fine particles of bone.

Materials – Semi-industrial FPC samples prepared by the Halifax method (Idler, 1968), from round herring (samples H_1, H_2), from cod filleting scrap (samples C_1, C_2, C_4), and from cod fillets (sample C_3) were used in present experiments. Two samples of FPC prepared from round herring on laboratory scale without use of polyphosphoric acid (samples H_3, H_4) were also used. All samples were first dried in a vacuum oven at 50 C for 2 days and kept in screw-capped jars. The moisture remained practically constant around 3.5% and was neglected in the calculations of ashes and densities. The fat content of the samples was below 0.1%. Reagent grade chloroform and methanol were used for the preparation of the flotation mixtures. A 25-ml pycnometer provided with precision thermometer, and a capped side arm was used for the determination of densities — pycnometer Type A, described in Weisberger (1959, p. 131–190). Reagent grade isopropyl alcohol was used as immersion liquid. The size of samples varied from 0.3 to 2 g depending on the amount of material available. The air bubbles were forced out through the side arm by gentle tapping. All weighings were done at 20.0 C. This was achieved by cooling the pycnometer 1 or 2 degrees below 20 C and then adjusting the liquid to the mark by removing the excess with a hypodermic syringe at the moment when the temperature reached 20.0 C.

From preliminary experiments in which flotation liquids of various densities and various types of glassware were used, the following method was devised.

Method for estimation of bone material in FPC – About 2 g of FPC is weighed accurately into a 50- or 100-ml tared beaker and about 25 ml of flotation liquid consisting of 80% chloroform and 20% methanol v/v is added from a washing bottle. A glass rod or spatula is used to break up any lumps and to facilitate the separation of protein particles from bone splinters. Almost instantaneously the bone material settles on the bottom whereas the protein particles rise to the surface. As soon as there is enough clear liquid between the two layers of solids (5–10 min) the protein layer is transferred by decantation to a 125-ml separating funnel, all protein particles being washed down with a stream of flotation liquid from a washing bottle. The contents of the

separating funnel are shaken and left to settle for about 5 min. The bone material that settles in the tip of the cone is added to the first bone material in the beaker by quickly opening and closing the stopcock, the bore of which has been widened. This operation is repeated once more; the surplus liquid from the beaker is decanted back into the funnel. The bone material is dried to a constant weight by placing the beaker on a hot plate (temp < 50 C to avoid spattering) under an air current. Complete drying is achieved in about 10 min. The protein residue may be quantitatively recovered by filtration through filter paper. A single determination of bone material thus can be performed in 50–60 min; however, one operator can process at least six samples simultaneously.

Results and discussion — Bone is a calcified connective tissue and contrary to the widespread belief it is not an immutable structure. The chemical and physical structure of bone vary continuously. Some of the variations are linked with age and size of fish, others are seasonal, caused by reproductive cycles or by fluctuations in food supply. They also can be accidental — caused by disease or starvation.

Even a cursory examination of fish skeleton shows that the degree of calcification of a bone differs from one part to another. The differences in porosity are considerable so that the porosity is used as an index for classification of bones into groups of compact, coarse cancellous, and fine cancellous bones. The porosity of bones creates problems in flotation and in the determination of density. Some of the bone cavities contain marrow, which is usually rich in lipids; other cavities are filled with air. Both the air and the lipids influence the buoyancy of bone particles. The methanol is added to chloroform for two reasons: it lowers the density of flotation liquid and has good wetting and penetrating properties, which facilitate the displacement of air from cavities.

Besides bone there are other hard and often mineralized tissues such as calcified tendons, cartilage, ligaments, epithelium, and scales that by their physical and chemical properties are similar to bone. Thus, the material separated by density is recognized as containing these materials although being arbitrarily defined as bone. Any such determination is specifically valid only for the species examined at a given age or season, and for particular ecological conditions. The data shown in Tables 1 and 2 must be regarded only as indexes of order of magnitude.

TABLE 1. Composition, ash content, and densities of protein and bone fractions of cod FPC.

Sample of FPC	Composition (%)		Ash (%) in			Density of		
	Protein	Bones	FPC	Protein	Bones	FPC	Protein	Bones
C^4	64.0	36.0	23.1	3.6	59.7	1.387	1.302	1.910
C^2	75.8	24.2	16.0	3.8	59.4	1.329	1.305	1.855
C^1	79.1	20.9	14.6	3.6	53.4	1.276	1.266	1.791
C^3	99.6	0.4	2.7	2.7	40.0	1.241	n.m.[a]	n.m.[a]

[a]Not measured.

A mixture of 80% chloroform and 20% methyl alcohol ($d^{20} = 1.355$) was used for the separation of bone material in FPC. Mixtures of higher and

TABLE 2. Composition, ash content, and densities of protein and bone fractions of herring FPC.

Sample of FPC	Composition (%)		Ash (%) in			Density of	
	Protein	Bones	FPC	Protein	Bones	Protein	Bones
H_4	86.1	13.9	9.2	3.8	44.2	1.312	1.744
H_3	87.8	12.2	9.7	4.3	42.9	1.310	1.781
H_1	91.1	8.9	9.1	4.7	51.4	1.304	1.730
H_2	91.3	8.7	9.2	4.9	49.9	1.308	1.762

lower densities were also tried. The separation of bone material from cod FPC was equally successful with pure chloroform and with mixtures containing up to 23% methyl alcohol ($d^{20} = 1.340$). The best separation of bone and protein particles in FPC prepared from whole herring was obtained with chloroform and 20–23% methyl alcohol (v/v). The protein separated with pure chloroform or chloroform containing less than 20% methyl alcohol has high ash content because it contains some of the partially mineralized tissue. On the other hand, when mixtures containing more than 23% methyl alcohol were used, the ash content of bone fraction decreased sharply because of the precipitation of some high density protein fractions. A sample of bone material separated from whole herring FPC with flotation liquid $d^{20} = 1.340$ was refloated in pure chloroform $d^{20} = 1.489$ (Table 3).

TABLE 3. Ash content in fractions of bone material separated from herring FPC with liquid $d^{20} = 1.344$ and then suspended in $d^{20} = 1.489$.

Fraction	% of separated bone material	Ash (%)	Rate of precipitation in chloroform (d = 1.489)
I	75.6	59.0	Immediate
II	15.2	42.4	Settles after 10 min
III	9.2	10.7	Floats

Table 3 illustrates the heterogeneity of the bone material in herring FPC and shows that pure chloroform in this case separates at least 9.2% less bone material than chloroform containing 20% methyl alcohol. With other samples pure chloroform separated 12–15% less bone material from herring FPC than did chloroform containing 20% methanol. With cod FPC samples, pure chloroform is an efficient flotation liquid as are its mixtures with methanol because of the higher density of cod bones (Table 1, 2).

The mineral content in bone material from cod is consistently higher than in that from herring (Table 1, 2). The sample C_3 is an exception — the FPC was prepared from "boneless" cod fillets and microscopical examination of the separated bone material showed that only few splinters of compact bone were present; the bulk consisted of connective tissues.

The content of bone material in cod FPC can be calculated from ash content of the sample using the following formula: 1.73 × ash% of FPC − 4.3 = bone material %. This formula is the equation of the line drawn through the points representing ash content of the FPC versus the content of bone material of the sample as determined by flotation (Table 1). More experimental data is needed for the establishment of a similar formula for herring FPC.

Even highly purified proteins contain certain amounts of chemically bound sulfur, phosphorus, calcium, magnesium, potassium, sodium, and other elements that cannot be separated without a complete destruction of the protein. This mineral residue or ash content varies with the nature of protein and the method of purification. On the average in dried samples it varies between 1.5 and 5%. The residual ash in protein of cod FPC after the separation of bones varied from 2.7 to 3.8% (Table 1) and 3.8 to 4.9% in protein from herring FPC. Microscopic examination showed only few bone splinters in the purified proteins. This indicates that the flotation can be successfully used for analytical purposes. The proposed method can be easily modified to suit particular working conditions and kinds of FPC or fish meals. It does not require complicated operations or apparatus and is adaptable to determinations in series.

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Determining Cortisol and Cortisone Simultaneously in Salmonid Plasma by Competitive Protein Binding

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FAGERLUND, U. H. M. 1970. Determining cortisol and cortisone simultaneously in salmonid plasma by competitive protein binding. J. Fish. Res. Bd. Canada 27: 596–601.

A method was developed for the simultaneous determination of nanogram quantities of cortisol and cortisone in 0.1-ml samples of salmonid plasma by competitive protein binding (CPB). The steroids were separated by thin-layer chromatography on silica gel and CPB analysis was performed with chicken serum in the presence of Florisil.

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IN THE STUDY of the secretion rate of cortisol in sockeye salmon (*Oncorhynchus nerka*) undertaken in this laboratory (Donaldson and Fagerlund, 1968, 1969; Fagerlund and Donaldson, 1969), a fluorimetric method (Donaldson et al., 1968) has been employed. Since the secretion rate studies have been extended to cortisone (Fagerlund and Donaldson, in preparation), a sensitive method was developed for the simultaneous determination of nanogram quantities of cortisol and cortisone in 0.1 ml of salmonid plasma using the competitive protein binding (CPB) technique. Murphy (1967) has studied many aspects of competitive protein binding of cortisol and cortisone.

Materials and the technique — Thin layer sheets were Eastman silica gel Chromagrams 6060 with fluorescent indicator and were used untreated.

Cortisol-1,2-^{3}H, specific activity 55.0 Ci/mM, and cortisone-4-^{14}C, specific activity 53.0 mCi/mM, supplied by New England Nuclear Corporation, Chigaco, Illinois, were used without purification. Purity was checked from time to time in the thin-layer chromatography (TLC) system used in the method.

Standards of cortisol and cortisone were made up separately into aqueous solutions containing 40 ng of steroid per milliliter.

Chicken serum was either obtained locally from 3-month-old male chickens[1] or purchased from Winley-Morris Co., Ltd,. Montreal, P.Q. The two sera gave slightly different curves with standard amounts of steroids, presumably because of differences in the natural steroid content. Serum was mixed with enough tritiated cortisol to give an activity of 100,000–300,000 cpm/ml and frozen in lots of 2 or 4 ml. When needed, a plasma lot was quantitatively diluted with water to give a 4% solution that could be kept for several days if refrigerated.

Florisil, 60–100 mesh (Fisher Scientific Company, Fair Lawn, N.Y.), was screened with Endecott's test sieves to obtain a particle range of 60–80 mesh, which gave a smaller deviation between duplicate determinations than the untreated material. Florisil was measured with spoons fashioned from 6-mm glass tubing by constricting and bending pieces of tubing so as to form

[1]Courtesy of Mrs B. E. March, Department of Poultry Science, University of British Columbia, Vancouver 8, B.C.

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a cup and a handle at 90° angle to the cup. Six measurements with one such spoon filled to the brim gave the mean and standard deviation 33.14 ± 0.66 mg. Murphy (1967) used similar spoons made of plastic.

Solvents were distilled slowly through a Vigreux column and from each gallon, a 100-ml forefraction and endfraction were discarded.

Plasma samples (0.1 ml) were diluted to 1 ml with distilled water and extracted with 5 ml of methylene chloride by shaking for 15 sec in 10–15-ml glass-stoppered centrifuge tubes. The tubes were centrifuged for 2 min and the aqueous layer removed by aspiration. Duplicate 1-ml portions of the extract were evaporated in conical centrifuge tubes of similar size by blowing jets of nitrogen gas over the solvent while warming the tubes to 45 C in a water bath.

The evaporated extracts were chromatographed in dichloromethane–methanol–water (150:9:0.5) (Quesenberry et al., 1965) on TLC sheets cut in half to provide 10 × 20-cm chromatograms. The extracts were transferred by rinsing four times with two drops of 10% methanol in dichloromethane to points 1 cm apart along a baseline across the longer dimension of the sheets and run in channels made by scoring the sheets between the spots. Cortisol and cortisone markers were applied at each edge and in the center of the sheet. Development time was 10–15 min. The markers were located under ultraviolet light and the corresponding areas of the channels containing plasma extracts were cut into 1 × 1-cm squares with scissors. The squares were dropped directly into 10–15-ml glass-stoppered tubes containing 1 ml of distilled water. At this stage, the samples could be stored refrigerated for 1 or 2 nights.

One ml of the CBG solution was added to each tube, thus giving a final serum concentration of 2%. The tubes were covered with aluminum foil and heated at 45 C for 5 min in a water bath to free the CBG from the steroids originally present. The tubes were cooled under a cold-water tap and then transferred to a coldroom kept at 0 C where the remaining steps of the procedure were carried out.

After a minimum of 10 min cooling, 80 mg of Florisil was added with the appropriate spoon to all tubes, which were then stoppered. The contents were mixed for 2 min by inverting all tubes simultaneously in a tube rack covered with a piece of plywood that had been padded with foam plastic.

The tube contents were allowed to equilibrate for 10 min or longer, during which time the Florisil settled; then 1 ml supernatant from each tube was added to 10 ml of Bray's solution (Bray, 1960) and radioactivity was monitored for 5 min in a Packard scintillation counter.

To determine recovery, a suitable aliquot of each of the dilute standard solutions of cortisol and cortisone was measured out and both were combined. The volume was made up to 1 ml with water and extracted with 5 ml of dichloromethane simultaneously with the extraction of a set of plasma samples. Three 1-ml aliquots of the extract were evaporated and chromatographed together with a set of duplicate aliquots of extracts of five plasma samples. The amounts of the chromatographed standards were chosen so as to fall within the anticipated range of steroid concentrations in the plasma extracts.

For the determination of standard curves, duplicate samples of blanks and of steroid standards chosen so as to cover the anticipated range of plasma steroid concentrations were diluted to 1 ml with water and CPB analysis of these standards was run simultaneously with the chromatographed plasma extracts and chromatographed standards.

The amount of steroid (nanograms/tube) obtained by plotting counts per minute on the standard curve was multiplied by 5 (if ⅕ of the total plasma extract was chromatographed) and corrected for mean recovery of the three chromatographed standards to give the number of ng/0.1 ml of plasma, which equals the number of μg/100 ml of plasma.

Evaluation of the method — Tritiated cortisol was found to be suitable for determination of both cortisol and cortisone. Standard curves for the two steroids (Fig. 1) indicated that cortisone was bound more firmly than cortisol to the CBG, resulting in somewhat more cortisol-^{3}H being replaced from the protein and consequently removed by Florisil from the solution.

Labelled cortisone was tried initially and gave standard curves similar to those obtained with labelled cortisol. When tritiated cortisol was used, 2 ml of a 2% plasma solution in the presence of 80 mg Florisil bound 77–82% of the label when no steroid was added. Tritiated cortisol and cortisone were more suitable than the ^{14}C-steroids because the higher specific activity of the former facilitated the use of high radioactivity in the samples without introducing large amounts of steroid.

The slope of the standard curve could be altered by varying the amounts of CBG and Florisil (Fig. 2). By choosing conditions that produced a standard curve with a steep slope, the effects of variability in the procedure on the precision were minimized.

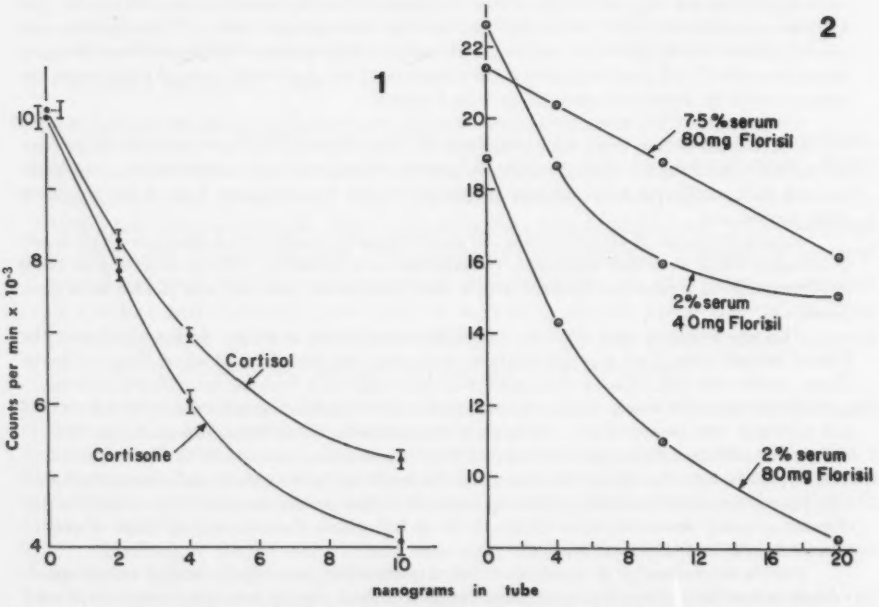

FIG. 1. Competitive protein binding of cortisol and cortisone standards. Each point represents mean and standard deviation of four determinations of duplicates.
FIG. 2. Effect of changes of the amount of Florisil and of dilution of serum on binding of cortisol.

Values for the proportional standard deviation S (Bundy et al., 1957) of eight duplicate determinations of various amounts of cortisol and cortisone standards (not chromatographed) were as follows:

Ng in tube	0.5	2	4	10
Cortisol	0.301	0.037	0.028	0.056
Cortisone	0.256	0.054	0.045	0.073

Those for duplicate determinations of chromatographed plasma samples were:

Ng in tube	0.2–1.9	2–5.9	6–10
Cortisol	0.177(18)	0.111(13)	0.136(15)
Cortisone	0.171(20)	0.113(20)	0.092(20)

Numbers of duplicate determinations are in parentheses. S values were obtained with the formulas $S = \sqrt{\frac{\Sigma\alpha^2}{2N}}$ and $\alpha = \frac{2(X_1 - X_2)}{X_1 + X_2}$ where N is the number of duplicates and X_1 and X_2 are results of duplicate determinations.

When greater accuracy is required in the low concentration range than that obtained (SD 17.7 and 17.1%), it is of advantage to take a larger aliquot of methylene chloride extract for chromatography or to use a larger amount of plasma and dilute with a proportionately smaller amount of water.

The R_f values of cortisol and cortisone markers did not change when the markers were added to plasma extracts. Extracted and chromatographed blanks were undistinguishable from unextracted and unchromatographed blanks.

The recovery of extracted and chromatographed standards when calculated from seven determinations of triplicate 4-ng samples was $87.4 \pm 10.2\%$ for cortisol and $78.3 \pm 4.0\%$ for cortisone. These values were similar to recoveries obtained when the steroids were eluted from the discs with 10% ethanol in dichloromethane rather than adding the discs directly to the CBG solution without prior elution of the steroid.

Cortisol values obtained by CPB assay of 32 samples of plasma (Fig. 3) compared favourably with the values obtained with fluorimetric assay (Donaldson et al., 1968) performed on duplicate 1-ml samples. Differences between values obtained with the two methods were less than 20% in 29 samples (90%) and less than 15% in 24 samples (78%).

Discussion — Murphy (1967) compared the relative affinity of several steroids to CBG in plasma from nine species of vertebrates. Of these, chicken plasma bound almost equal amounts of cortisol and cortisone. Chicken CBG, was, therefore, chosen for use in the procedure described here. The same author found that Florisil absorbed very little tritiated cortisol, but this latter finding was not substantiated in the present study.

The standard deviation of points on the standard curves, when determined on separate occasions (Fig. 1), was greater than the standard deviation of duplicate determinations, which may be derived for these points from the first table in the text. Therefore, when maximum accuracy was required, curve standards were included with each set of plasma samples to be analyzed. However, plasmas were grouped so that the anticipated steroid values fell within as narrow a range as possible to minimize the number of standards needed. For routine determinations, a curve was drawn from the means of standards from several runs, using identical aliquots of pooled chicken serum containing tritiated cortisol.

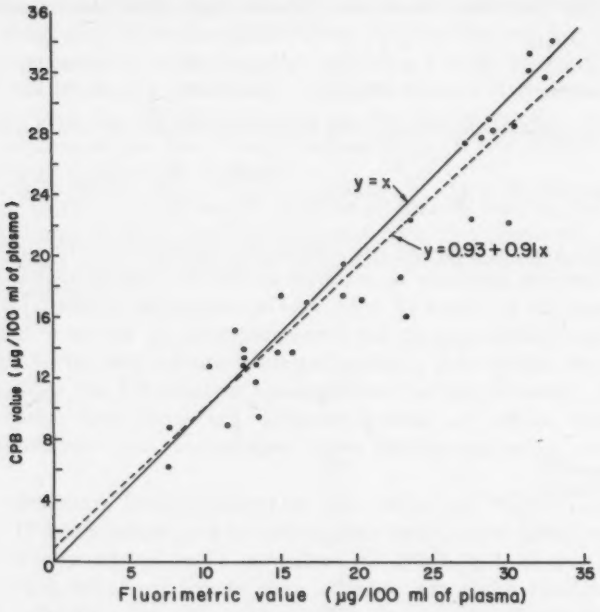

FIG. 3. Regression of CPB values on fluorimetric values (broken line), obtained from 32 duplicate cortisol determinations.

Cortisol and cortisone are the major corticosteroids in the plasma of sockeye salmon (Idler et al., 1959; Schmidt and Idler, 1962). Progesterone and 17α-hydroxyprogesterone, which are also present in considerable amounts, have higher R_f values than cortisol and cortisone in the solvent system used in the present work (Quesenberry et al., 1965) and, therefore, do not interfere. 20β-Dihydro-17α-hydroxyprogesterone (Schmidt and Idler, 1962) and 20β-dihydrocortisone (Idler et al., 1962) have been found as minor steroids in salmon plasma and have R_f values close to that of cortisol. These steroids did not interfere with the protein binding of cortisol-^{3}H under the conditions of the assay when tested in concentrations of 8 ng per assay tube, equivalent to 40 µg/100 ml of plasma.

Cortisol and cortisone concentrations in sockeye salmon determined with the CPB method will be reported in future communications (Fagerlund and Donaldson, in preparation). In resting 16-month-old juvenile coho salmon held in fresh water, plasma cortisol concentrations were found to be 0 µg/100 ml and cortisone concentrations 3.68 ± 3.93 µg/100 ml. After the fish had been agitated with a net for 30 min the concentrations were 18.88 ± 5.31 and 3.76 ± 1.75 µg/100 ml respectively.

Acknowledgments — The technical assistance of Mr W. Hadaway is gratefully acknowledged.

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Scallop Sterols are Hypocholesterolemic Relative to Cholesterol for the Chick

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IDLER, D. R., L. M. SAFE, G. A. MACKINNON, AND F. G. PROUDFOOT. 1970. Scallop sterols are hypocholesterolemic relative to cholesterol for the chick. J. Fish. Res. Bd. Canada 27: 601–603.

Groups of 8-day-old chicks were fed 14 days on a standard diet, or on a standard diet supplemented with scallop sterols or cholesterol, or both. The blood cholesterol concentration increased significantly ($P < .01$) when .5% cholesterol was incorporated into the diet and there was a possible decrease ($.05 < P < .10$) when .5% scallop was fed.

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MOLLUSC STEROLS generally contain a complex sterol mixture relative to many other marine invertebrates. Among the edible molluscs, scallops are of considerable economic significance and are eaten in quantity. In recent studies marine sterols have been shown to produce hypocholesterolemia in chicks (Reiner et al., 1960, 1962). Towards a further evaluation of this phenomenon it was decided to investigate the possible hypocholesterolemic effects of scallop sterols.

Methods — Fresh scallop muscle was ground and extracted with hot acetone. The oil was saponified using 10% methanolic potassium hydroxide and the neutral fraction yielded a white crystalline sterol mixture. Gas–liquid chromatography (GLC), carried out on the steryl trimethylsilyl ethers (Idler and Wiseman, 1968), confirmed the major components of the mixture to be cholesterol 25%; 22-dehydrocholesterol 12%; brassicasterol 19%; 24-methylenecholesterol 20% (Wainai et al., 1964).

Groups of 8-day-old leghorn cockerels were fed ad libitum on a standard diet, or on a standard diet supplemented with cholesterol or scallop sterol, or both. On the 14th day of test feeding approximately 2 ml of blood was removed from the jugular vein of each chick. Serum cholesterol concentration was measured according to the method of Sperry and Webb (1950). Analysis by GLC showed that approximately 99% of the serum sterols were inseparable from cholesterol.

Results — Serum cholesterol levels resulting from the four rations are shown in Table 1. The presence of .5% cholesterol in the chick diet produced a highly significant increase in chick serum cholesterol (Table 2). A possibly significant decrease in serum cholesterol ($.05 < P < .10$) occurred when .5% scallop sterol was fed. There was no significant interaction between the dietary cholesterol and scallop sterol. The mean values of serum cholesterol were 132 mg/100 ml for the chicks on the standard diet with scallop sterol, 138 for the standard diet alone, 150 for the standard diet with both cholesterol and scallop sterol, and 162 for the standard diet with cholesterol (Table 1).

TABLE 1. Serum cholesterol levels of chicks on sterol supplemented diets.

	Standard diet[a]	Standard + .5% cholesterol	Standard + .5% scallop sterol	Standard + .5% scallop sterol + .5% cholesterol
No. of chicks	14	14	14	14
Serum cholesterol (mg% ± SE)	138±4	162±4	132±6	150±6
Mean food consumption (g/chick per day) days 8–21	21.8	22.2	23.5	19.0
Mean wt gain (g/chick) days 8–21	168	164	156	153

[a]Cornmeal 45%; ground wheat 14%; stabilized tallow 2%; soybean meal 35%; ground limestone 1.5%; dicalcium phosphate 1.5%; iodized salt .5%; micronutrients .5%.

TABLE 2. Analysis of variance showing the effects on serum cholesterol of incorporating cholesterol and scallop sterol into a chick ration.

Source	df	Mean square	F	Significance
Cholesterol	1	6090	16.45	HS* ($P < .01$)
Scallop sterol	1	1441	3.89	PS ($.05 < P < .10$)
Cholesterol + scallop sterol	1	8	<1	NS ($P > .10$)
Error	52	370.2		

*HS = highly significant; PS = possibly significant; NS = nonsignificant.

Discussion — When scallop sterol was fed there was no increase in serum cholesterol, even though cholesterol is a significant component (25%), and there may have been a decrease ($.05 < P < .10$).

In the present work when the chick diet was supplemented with 0.5% cholesterol the serum cholesterol level increased by 17%. This increase is statistically highly significant but is much lower than the increases produced when 1% cholesterol was fed (Reiner et al., 1960, 1962). In this study .5% cholesterol and .5% scallop sterols were used because the amount of diet the chicks would eat was overestimated.

A sterol mixture of butter clam (*Saxidomus giganteus*) was shown to exert a hypocholesterolemic effect in chicks (Reiner et al., 1960). As a sequel to this experiment, Reiner et al. (1962) fed sterols from two different algae, one consisting mainly of fucosterol and the other of a mixture of fucosterol and sargasterol. Both sterol mixtures caused a diminution in plasma cholesterol. In the present study the elevation of plasma cholesterol by exogenous cholesterol (162 mg%) was insufficient to permit us to establish that the depression of this effect by scallop sterol (150 mg%) was significant. In a future experiment sterol will be supplemented at the 1% level.

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Occurrence of Trigonelline (N-Methyl Nicotinic Acid) in the Adductor Muscle of a Lamellibranch, the Sea Scallop (*Placopecten magellanicus*)

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HILTZ, D. F. 1970. Occurrence of trigonelline (N-methyl nicotinic acid) in the adductor muscle of a lamellibranch, the sea scallop (*Placopecten magellanicus*). J. Fish. Res. Bd. Canada 27: 604–606.

Trigonelline, associated with its isomer homarine, has been found in amounts ranging from 0.7 to 1.3 μmoles/g in scallop adductor muscle. Separation of the two betaines was achieved by column chromatography on a cation-exchange resin, using a linear gradient of 2 N HCl into H_2O. Identification of trigonelline was based on spectral properties and on behavior in thin-layer, paper, and column chromatography.

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IN A PREVIOUS STUDY, Hiltz and Dyer (1970) found appreciable amounts of nonnucleotide, ultraviolet-absorbing material, with maximum absorption at 272 nm, in neutralized perchloric acid extracts of the adductor muscle of scallop. This material appeared in the initial peak obtained during the loading and water-washing of extracts on Dowex-1 (formate) chromatographic columns prior to the elution of nucleotides. The absorption maximum (272 nm) suggested homarine (N-methyl betaine of picolinic acid), but the absorption ratios were not typical and suggested contamination with other compounds. The fraction containing this material was rechromatographed on a small (13 × 1 cm) column of AG 50W × 8 (H^+) resin (Calbiochem, Los Angeles), using a linear gradient of 2 N HCl (500 ml) into H_2O (500 ml). Three peaks were obtained (Fig 1). Material not held by the resin appeared in peak A; homarine, now having spectral properties identical with those of the pure compound, appeared in peak B; finally, another, unexpected substance, well separated from the homarine fraction, was eluted, giving rise to peak C. These results were observed consistently in several chromatographic runs of scallop muscle examined both immediately post-mortem and after 6 days in ice.

Fractions common to peak C were pooled, dried in a rotary evaporator, and reconstituted in a minimum amount of water for spectrophotometric and chromatographic examination. The absorption spectrum (*p*H 1) showed two wavelength maxima — a major peak at 264–265 nm and a minor one at 271 nm — and a minimum at 240 nm, with little change at neutral or alkaline *p*H. These properties suggested a pyridine monocarboxylic acid derivative (Green and Tong, 1956), possibly the N-methyl betaine of nicotinic acid, or trigonelline. Trigonelline (Nutritional Biochemicals, Cleveland, Ohio), chromatographically homogeneous in the solvents listed below, had identical

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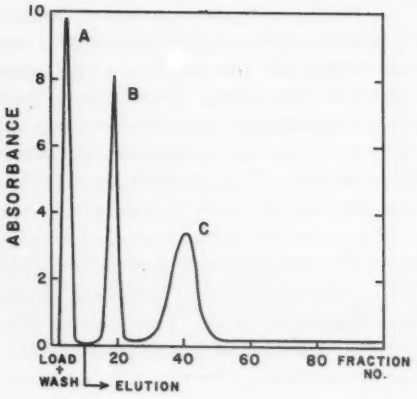

FIG. 1. Column chromatographic separation of the betaines homarine and trigonelline in scallop adductor muscle immediately post-mortem. A nonnucleotide fraction from $HClO_4$ extracts, having maximum absorption at 272 nm, eluted initially from Dowex-1 (formate) columns with water, was rechromatographed on AG 50 W resin. Fraction size was 10 ml. Absorbance (arbitrary units) was monitored by an ultraviolet absorption meter, output band 20 nm wide, peaked at 265 nm. Scallop muscle iced for 6 days gave similar results.

TABLE 1. R_f values of some pyridine carboxylic acids and their derivatives on silica gel layers. Solvent: 1 M ammonium acetate/ethanol (2:8). Detection: shortwave ultraviolet light.

Compound[a]	R_f
Picolinic acid	0.64
Quinolinic acid	0.11
Nicotinic acid	0.90
Nicotinamide	0.90
1-Methyl nicotinamide	0.22
N-Methyl picolinic acid (homarine)	0.42
N-Methyl nicotinic acid (trigonelline)	0.29
Peak C (Fig. 1)	0.29

[a]Obtained from Nutritional Biochemicals Corporation, except 1-methyl nicotinamide (Sigma) and homarine (Aldrich).

spectral characteristics; the E at 264 nm was 3800 in 0.1 N HCl, and 3700 at *p*H 7 and in 0.1 N NaOH. Thin-layer chromatograms on Merck precoated plates of silica gel F_{254} (Brinkman Instruments, Rexdale, Ont.), developed with 1 M ammonium acetate/ethanol (2:8) (Diamond and Levy, 1967) and viewed under shortwave ultraviolet light, showed the unknown substance and trigonelline to have identical R_f values; this particular solvent system was effective in resolving several pyridine carboxylic acids and their derivatives (Table 1). The unknown also ran in the position of trigonelline on thin-layer chromatograms in propanol/10% NH_3 (Bollinger, 1965), and on paper chromatograms in isopropanol/acetic acid/water (Beers, 1967). Column chromatography of mixtures of homarine and trigonelline, and of these plus added unknown, gave elution patterns identical with Fig. 1. On the foregoing evidence, the substance eluted as peak C is identified as trigonelline.

Of the naturally occurring quaternary ammonium compounds, homarine is known to be widely distributed among invertebrate species (Gasteiger et al., 1960), but the presence of its isomer, trigonelline, has been less frequently reported (see Beers, 1967). Beers found appreciable quantities of trigonelline (4–13 μmoles/g) in certain marine crabs and in lobster, but did not detect it in two species of molluscs examined. The present note appears to be the first record of its occurrence in the Lamellibranchiata. The concentration ranged from 0.7 to 1.3 μmoles/g of muscle in several lots of scallops examined, and was unaffected by postmortem storage in ice (up to 6 days). It was always less than the concentration of homarine (1.6–2.6 μmoles/g) (Hiltz and Dyer, 1970), as was found by Beers for Crustacea. The possible role of these betaines and associated nitrogenous compounds in osmotic regulation in invertebrates has been discussed by Florkin (1966).

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Relationship Between Growth Rate and Ribonucleic Acid Concentration in Some Invertebrates[1]

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SUTCLIFFE, W. H., JR. 1969. Relationship between growth rate and ribonucleic acid concentration in some invertebrates. J. Fish. Res. Bd. Canada 27: 606–609.

A positive relationship between growth rate and ribonucleic acid concentration is shown for 24 species, mostly arthropods and microorganisms. It is suggested that such a relationship might exist also for mammals.

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IT HAS BEEN PROPOSED THAT concentration of ribonucleic acid (RNA) can be used as a growth indicator in invertebrates (Sutcliffe, 1965). A positive correlation between RNA concentration and growth rate has been well documented for microorganisms (e.g., Maaløe and Kjeldgaard, 1966; Leick, 1968), but it remains to be shown whether a consistent relationship exists with other organisms. This presentation provides some evidence that a growth rate–RNA relationship exists not only for microorganisms but also in some preadult growing invertebrates.

Methods — Cultures of the cockroach *Blatella germanica* were maintained at 28–30 C on a diet of Purina Cat Chow. Single females with egg cases were isolated to produce small colonies of young of the same age. At intervals of 5–8 days, two to five young were killed by freezing and were vacuum dried. Each age-group, after weighing, was analyzed for RNA by the orcinol method of Schneider (1957) as used by Sutcliffe (1965). Centrifuging for washings was done at about 2800 *g*. Loss of low molecular weight RNA may have occurred but the error appears to be constant. Growth, as percent per day, was computed by:

$$\%/\text{day} = \left[\exp\left(\frac{\ln W_t - \ln W_0}{t - t_0}\right) - 1\right] \times 100$$

where W_t = dry weight at any time t (in days) and W_0 = dry weight at t_0 previous to t.

Some measurements were made on two other organisms. The carnivorous pteropod *Clione limacina* was grown at about 15 C and fed a smaller species, *Spiratella retroversa*. The shrimp *Periclimenes americanus* was maintained at about 27 C and fed bits of fresh bivalve meat. Pteropods and shrimp were frozen, vacuum dried, and analyzed as above.

Results and discussion — Growth versus RNA concentration for the above analyses and for data from the literature is presented in Fig. 1. In all cases the information was taken from growing or preadult organisms. Nearly all the data from other work were from estimations by the orcinol reaction, although extraction methods varied widely. In the presentation of data of other workers, an attempt was made to show the range of values encountered rather than all given points. For example, although Leick (1968) gives data on 15 microorganisms, three points are presented here representing the fastest growing organism, the slowest, and one in between. Where rate was given in doublings per hour, this was equated with the other material by multiplying by 24 hr and 100. Where water and protein content were not given, values from Altman and Dittmer (1964) were used. A protein content of 60% dry weight was assumed for Leick's microorganisms. The assembled data represent 24 species, mostly microorganisms and arthropods. It should be noted that the manner of presentation of data in this report is one of convenience; no theoretical conclusions are intended, for the primary relation lies between RNA and protein.

It is seen that although there is a considerable spread, attributable in part to differences in method, a definite relationship between growth rate and RNA exists. The shape of the curve differs from that given by Sutcliffe (1965) apparently because extrapolation was made from too few points in the previous work, but absolute values do not differ greatly within the ranges

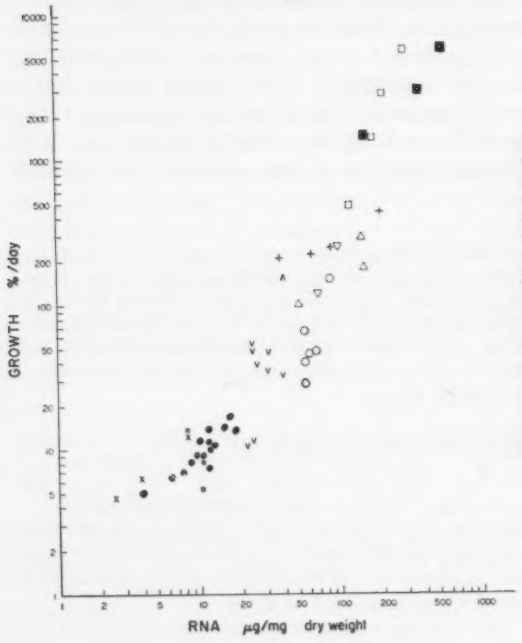

FIG. 1. Growth rate in percent per day versus RNA concentration: ● cockroach *Blatella germanica*; × amphipod *Talorchestia platensis* (Sutcliffe, 1965); ∗ shrimp *Periclimenes americanus*; ∨ pteropod *Clione limacina*; ○ flour beetle *Tribolium confusum* (Devi et al., 1963); △ flagellate *Polytomella caeca* (Jeener, 1953); ▽ mosquito *Aedes aegypti* (Lang et al., 1965); ∧ blowfly *Calliphora erythrocephala* (Price, 1965); + fruit fly *Drosophila melanogaster* (Church and Robertson, 1966); □ bacterium *Salmonella typhimurium* (Maaløe and Kjeldgaard, 1966); ■ 15 microorganisms (Leick, 1968).

used. Pease (1968), on the other hand, found no consistent relation between RNA and increase in dry weight of the brine shrimp *Artemia salina*.

In arthropods for which information is available (Church and Robertson, 1966; Devi et al., 1963; Lang et al., 1965), upon reaching the adult stage the RNA concentration tended to level off at some fraction of that attained during actively growing stages. In mammals, the data of Munro and Gray (1969) showing a correlation between RNA concentration in tissues and size might also be interpreted as a function of growth rate. Figure 2 shows their data on RNA concentration in the liver together with percent growth during the 1st week after birth (from Altman and Dittmer, 1964). The trend is evident even though the RNA information is from a single tissue and adult animals.

After the above work was completed, the work of Dagg (1969) came to the author's attention. Dagg found significant correlations between RNA concentration and growth of the copepod *Euchaeta japonica* and the brine shrimp, although there was considerable scatter in the brine shrimp data and the slopes of the fitted curves differed in the two organisms. If Dagg's curves are plotted with the data given here, but excluding those analyses carried out for this paper, a linear trend is given on the double log plot and crossing the 1%/day growth level near 10 µg RNA/mg dry weight. The difference might be accounted for by systematic loss of RNA as indicated previously. Dagg used the Schmidt-Thannhauser technique as modified by Munro and

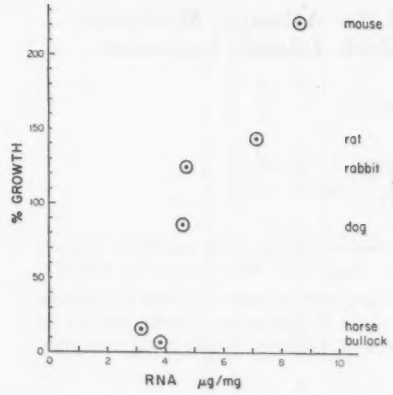

FIG. 2. Percent growth in first week after birth (Altman and Dittmer, 1964) versus RNA content of fresh liver (Munro and Gray, 1969).

Fleck (1966). Although the differences do not invalidate the material presented in this paper, comparisons are now being made with the two methods, which we hope to present later. Pease (1968) and Dagg agree that RNA cannot be used for growth estimates as presented by Sutcliffe (1965), and indeed there is considerable scatter within individual species as can be seen in Fig. 1.

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Northern Range Extension of the Atlantic Mackerel, *Scomber scombrus*, to Black Island, Labrador

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PARSONS, L. S. 1970. Northern range extension of the Atlantic mackerel, *Scomber scombrus*, to Black Island, Labrador. J. Fish. Res. Bd. Canada 27: 610–613.

During August 1969, 785 specimens of *Scomber scombrus* were captured in southern Labrador coastal waters, extending the known range in the western North Atlantic to Black Island, Labrador, more than 50 miles north of Triangle Harbour, the previously authenticated northern range limit.

Received October 20, 1969

ATLANTIC MACKEREL, *Scomber scombrus* (Linnaeus) 1758, are known to occur along the eastern coast of North America from Cape Hatteras off North Carolina to Triangle Harbour, Labrador, but they are usually not abundant north of the southern Gulf of St. Lawrence or Newfoundland (Leim and Scott, 1966). Goode (1884) gave the northern-most range of *Scomber scombrus* as 56°N but this claim was based on hearsay evidence and has not been confirmed by authentic records of capture. Mackerel have been reported sparingly several times from the Strait of Belle Isle area (Storer, 1853; Weiz, 1866; Brown, 1872; Grenfell, 1913; Anon., 1933). Stearns (1884) reported the occurrence of mackerel at Triangle Harbour (52°50′N) on the southern Labrador coast. Although Sette (1950) states that the authenticated range extends no farther north than the Strait of Belle Isle, Leim and Scott (1966) give Triangle Harbour as the northern range limit. The present records document the occurrence of mackerel north of the Strait of Belle Isle and represent an extension of the species range in the western North Atlantic to Black Island (53°46′N), more than 50 miles north of the previously authenticated northern range limit.

During a cruise by the Fisheries Research Board of Canada vessel *Investigator II* to the southern Labrador area from August 7–20, 1969, to conduct herring investigations, 785 mackerel were caught in anchored gillnets set in coastal waters over depths of 10–30 fath, with surface temperatures from 6.7 to 13.7 C (Table 1). Each set consisted of a string of five nylon herring nets of different mesh sizes (2 inch, 2¼ inch, 2½ inch, 2¾ inch, 3 inch), 90 fath in length and 3 fath deep; these nets were generally set 1 fath beneath the surface. The length of time the nets were set ranged from 16 to 68 hr.

One hundred and ninety mackerel were taken north of Triangle Harbour. The northern-most record was one specimen obtained in a set near Black Island (53°46′N). These specimens ranged in total length from 32.1 to 45.4 cm, with an average total length of 38.5 cm. Seventy-six of the 188 mackerel examined for sex and stage of sexual maturity were males, and 112 were females.

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TABLE 1. Catch records of *Scomber scombrus* in southern Labrador coastal waters on the *Investigator II*, cruise 6, August 7–20, 1969.

Station no.	Date of capture	N lat	W long	Locality	Temp (*C*)			No. of specimens	Total length (*cm*)	
					Surface	10 m	20 m		Mean	Range
37	Aug. 8	51°41′	56°29′	Carrol Cove, Strait of Belle Isle	6.7	1.1	0.6	13	...	...
39	11	52 17	55 43	East Side, Club Cape, St. Lewis Inlet	12.2	4.0	0.5	12	39.4	34.2–43.1
40	11	52 18	55 45	West Side, Club Cape, St. Lewis Inlet	12.3	2.3	0.8	6	39.1	36.7–42.1
41	11	52 17	55 37	Trap Cove, Great Caribou Is.	12.9	4.5	1.1	43	38.4	33.4–45.0
42	12	52 38	55 46	Ship Hbr.	11.0	6.5	2.4	148	37.2	33.1–45.0
43	12	52 41	55 52	George's Cove, St. Michael's Bay	12.1	7.0	3.0	373	38.9	31.5–46.4
44	13	53 01	55 49	Stoney Is., Hawke's Bay	13.7	7.5	2.0	168	37.5	32.1–44.8
46	14	53 21	53 47	Open Bay Pt., Porcupine Bay	10.5	10.0	4.0	5	40.6	37.2–45.4
47	14	53 24	54 47	Batteau Pt., Sandy Bay	10.0	10.0	5.0	5	37.6	35.1–39.9
48	15	53 33	55 55	Deer Is., Indian Tickle	9.7	10.0	6.0	11	39.4	36.9–44.4
50	18	53 46	56 22	Black Is.	9.4	4.2	1.8	1	...	...

As far as could be determined from gross visual examination of the gonads, all were in immature condition; most, however, appeared to be recovering from a previous spawning. Meristic data on these specimens fall within the limits cited by Leim and Scott (1966) and MacKay and Garside (1969).

According to MacKay (1967), mackerel spawn in June and July in the southern Gulf of St. Lawrence over the Magdalen Shallows. After spawning they disperse throughout the Gulf, being more concentrated in the northern part in August. The occurrence of mackerel in the Strait of Belle Isle and north along the southern Labrador coast to Black Island probably represents a northward extension of this postspawning dispersal and migration.

The author had opportunity, during the cruise on which these specimens were captured, to interview fishermen at several fishing communities from Red Bay to Domino. Their observations indicated that mackerel were unusually abundant in southern Labrador coastal waters during August 1969. Apparently, larger than usual quantities were caught, both in cod traps and in herring nets set for bait.

Sette (1950) cites observational evidence that indicates that the Atlantic mackerel in the western North Atlantic prefers temperatures above 8 C, although it appears to frequently tolerate temperatures down to 7 C; it is only rarely found in temperatures as low as 4 or 5 C. Bathythermograph data obtained at the time of our catches indicate that temperatures of 7 C and higher were generally restricted to the upper 10–12 m or less, and at nearly half the stations did not occur deeper than 5 m.

Templeman and Fleming (1953) associate an increase in mackerel catches along the east coast of Newfoundland from the mid-1940's onward with a recent period of climatic warming. Unfortunately, the temperature information available for other years for southern Labrador coastal waters is inadequate for a valid comparison with 1969 temperatures. Nonetheless, it is probable that higher than usual temperatures in the inshore surface layers are at least partly responsible for the apparent unusual abundance of mackerel along the southern Labrador coast during the summer of 1969.

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Vital Statistics of a Population of Great Lakes Rainbow Trout (*Salmo gairdneri*) Characterized by an Extended Spawning Season

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Adult fish moved into Bothwell's Creek, Lake Huron, between October 29 and May 3. Run A (October 29–February 15) was composed of 640 fish, run B (February 16–May 3) of 610 fish. Run A spawned between December 29 and February 14 and left the river. Run B spawned between February 20 and April 30. Total lengths of males ranged from 31 to 80 cm, females from 21 to 90 cm. Fourteen categories of life history were identified within the population of which 99% had become smolts after 2 years of stream life. Fish of age 5 were dominant. Differences in age class structure and size of fish occurred between runs A and B. Sea lamprey scars were evident on 8.6% of fish, all over 46 cm in total length.

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RAINBOW TROUT, *Salmo gairdneri* Richardson, first introduced to the Great Lakes late in the 19th century (U.S. Bureau of Fisheries, 1905), are self-sustaining through natural reproduction in tributary streams. Although adults may move into the lower reaches of a number of streams in late autumn, it has been generally believed that the spawning of wild fish in Ontario waters

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occurs only during the spring months (MacKay, 1960). However, recent studies have revealed that both winter and spring spawning occurs in Bothwell's Creek, flowing into Lake Huron at Leith, Ont.

This note documents details on the migration and extended spawning of the Bothwell's Creek population and analyzes differences in the vital statistics of winter- and spring-spawning fish.

Materials and methods — Bothwell's Creek (43°30′N, 80°45′W) is 42 km in length including tributaries, and drains an agricultural watershed of 50 km² at an average gradient of 16.5 m/km. Spring sources maintain a minimum flow of about 0.14 m³, and maximum freshet discharges may reach 5.7 m³. Numerous spawning beds are dispersed over the stream system.

The numbers and distribution of adult fish in the system were recorded weekly by visual observation from the stream bank between October 29, 1965, and May 1, 1966. Biological data were gathered weekly on trout removed by dipnets.

Age was estimated by the examination of scales (Hartman, 1959), life histories were categorized (Shapovalov and Taft, 1954), and rates of growth were calculated by a linear semilogarithmic regression of weight on total length (Ejekiel and Fox, 1959). Evidence of sea lamprey predation was noted. Released fish were identified with colour-coded Atkins tags for subsequent visual identification during weekly inspections of the stream system. All data were examined for similarities and differences between the movement and vital statistics of fish in winter (run A) and spring runs (run B.)

Migration — Adult rainbow trout, comprising the 1965–66 spawning population, moved into the stream between October 29 and May 3. Because of two obvious peaks in the numbers of upstream migrants, separated by a period of no new arrivals between February 2 and 15, the upriver migration was divided into run A (October 29–February 15) composed of 640 fish (176 sampled), and run B (February 16–May 3) composed of 610 fish (171 sampled).

Spawning — Spawning within run A extended from December 29 to February 14 and reached a maximum between January 15 and February 5; and within run B extended from February 20 to April 30, reaching a maximum between March 16 and April 16. There was a total exodus of run A trout from the stream before the arrival of run B, no fish from run A being captured or seen after February 15.

Age and maturity — Both sexes matured first at age 3. All males were mature by age 4, although some females did not reach maturity until age 5.

Maiden fish were twice as numerous in run B as in run A, but only 52% of run B were repeat spawners compared with 75% in run A. Males were significantly fewer in run B than in run A. The larger part of run A was fish between ages 5 and 7 (74%), whereas run B consisted largely of fish between ages 3 and 5 (79%).

Life history categories — Fourteen categories of life history were identified in the population, over 99% of which had spent 2 years in the stream as parr (Table 1). The youngest fish, 3 years old (14% of the spawning population), had spent less than 1 year in the lake before first spawning. The oldest fish were of age 8, having spent between 1 and 2 years in the lake before first

TABLE 1. Percentages of the spawning population of rainbow trout in Bothwell's Creek in different age categories.

Category[a] (*stream/lake*):	1/1.2S	1/2.1S	2/1S	2/2S	2/3S	2/4S	2/1.1S	2/1.2S	2/1.3S	2/1.4S	2/1.5S	2/2.1S	2/2.2S	3/1.1S
Run A : Oct. 29, 1965–Feb. 15, 1966														
Males ($n=93$)	0	0	6.5	1.0	1.0	0	15.0	42.0	29.0	4.3	1.0	0	0	0
Females ($n=80$)	0	0	12.5	0	0	0	16.2	35.0	17.5	17.5	1.2	0	0	0
Subtotal	0	0	9.2	0.6	0.6	0	15.6	38.8	23.8	10.4	1.1	0	0	0
Run B : Feb. 16–May 1, 1966														
Males ($n=62$)	0	0	29.0	4.8	8.0	0	37.0	16.1	6.5	0	0	0	0	0
Females ($n=105$)	1.0	1.0	13.3	4.7	4.7	5.7	21.0	31.4	9.5	1.0	1.0	1.0	4.7	1.0
Subtotal	0.6	0.6	19.2	4.8	6.0	3.6	27.0	25.8	8.4	0.6	0.6	0.6	3.0	0.6
Total ($n=340$)	0.3	0.3	14.0	2.3	3.3	1.7	21.3	32.9	16.0	5.8	0.9	0.3	1.5	0.3

[a] / — separates years of stream life from lake life; . — subdivides lake life into years before maturation (to the left of the period) and number of spawnings followed by an S (to the right of the period).

spawning. Fish spending less than 1 year in the lake before first spawning constituted 34% of run B, more than twice as numerous as in run A where the dominant groups had between 1 and 2 year's lake residence before spawning.

Growth — Mature males ranged in length from 31 to 80 cm with females between 21 and 90 cm. Modal classes in length distributions, for males at 56–60 cm and females at 66–70 cm, resulted from the dominant age-group, age 5, which comprised 73 and 68% of the modes for males and females, respectively.

Sizes of male fish were similar for both runs, but female fish between 21 and 45 cm were absent in run B. Modes in length distribution for males and females of run A were 51–55 cm, and 71–75 cm, respectively; and in run B, 61–65 cm and 66–70 cm, respectively. No significant differences were evident between slopes of regression lines of weight on length calculated for each sex for each run, nor between runs. The average size of fish from run B was 5.5 cm longer and 0.34 kg heavier than from run A.

	No. fish		
	Run A (Oct. 29–Feb. 15)	Run B (Feb. 16–May 3)	Total
Maiden fish	44	82	126
Repeat spawners	132	89	221
Sex ratio	117/100	58/100	88/100
Age-groups			
3	16	24	40
4	28	55	83
5	70	56	126
6	42	26	68
7	18	2	20
Avg length (*cm*)	56.5	62.0	59.2
Avg wt (*kg*)	2.32	2.66	2.51

Lamprey parasitism — Sea lamprey (*Petromyzon marinus*) scars, which were observed on 30 fish, occurred more frequently on females (9.4%) than males (6.3%). Scars were absent on fish less than 46 cm in total length.

Discussion — The growth of trout breeding in Bothwell's Creek compares favourably with that recorded for an anadromous population within the native range (Shapovalov and Taft, 1954) and no doubt reflects an adequate food supply within a suitable stream and lake environment. The preponderance of 2-year-old smolts (99%) may also be a factor as Shapovalov and Taft (1954) and Hartman (1959) have both related a longer parr life to subsequent lake growth.

No other study has shown such a high a proportion of 2-year-old smolts. Shapovalov and Taft (1954), Chapman (1958), Greeley (1933), and Hartman (1959) all reported that at least 10% of migrants were fish of 1, 3, and 4 parr-

years. Neave (1940) stated that over half of the rainbow trout in his British Columbia studies migrated to the sea as yearlings, whereas Withler (1966) found the dominant age of downstream migrants to be 3 parr-years in other streams of that province. The dominance of a history of 2 years of early stream life among adults returning to Bothwell's Creek does not necessarily infer that all young fish leaving Bothwell's Creek are of this age. Rather, it may be that migrating young-of-the year and yearling fish suffer heavy mortality by bird or fish predation, or from other causes, during or after movement to the lake. However, age at maturity was similar to that for fish of other waters (Hartman, 1959; Shapovalov and Taft, 1954; Chapman, 1958).

A similar dominance of trout returning to Great Lakes streams after between 1 and 2 years of lake life was reported for Lake Michigan (Greeley, 1933). However, Hartman (1959) observed that Finger Lakes trout in New York usually spent less than 1 year in the lake, and Withler (1966) noted that British Columbia trout typically remained in the ocean for 2 years before reappearing in the rivers as mature adults. Repeat spawners (65% of migrants) were more numerous in Bothwell's Creek than reported by Chapman (1958) and Withler (1966) for saltwater populations (20%), or by Hartman (1959) for the Finger Lakes (33%).

The relative abundance of repeat spawners in Bothwell's Creek would seem to suggest a low mortality after the first postspawning period and may be related to the relatively close proximity of the river spawning beds to the lake. The average length of adult migrants in Bothwell's Creek, 59.2 cm, exceeds that of fish in all other waters recorded in the literature, except for Lake Michigan in 1932 (Greeley, 1933) at a time when lampreys were not yet abundant there. As growth is similar to that generally observed for other waters, the comparatively high average length reflects the ability of numerous adults to survive in the Owen Sound Bay complex to ages 5–7 years, with some fish reaching 8 years of age.

In Bothwell's Creek, the rainbow trout appear to migrate and spawn at two different times, with little or no mixing between the two groups. Also, certain basic differences in age and life history categories existed between fish in the two runs, run B having more repeat spawners, considerably fewer females, and more life history categories of 2/1S–2/4S than run A. As males mature more quickly than females, these differences may be related to the time required for fish to reach maturity. Both Shapovalov and Taft (1954) in California and Hartman (1959) in New York have previously observed a significant relation between sex ratio and time of spawning, although the duration of the spawning seasons were considerably less.

The extended spawning season in Bothwell's Creek, characterized by two separately migrating populations, is evidence of the plasticity of the rainbow to adapt migration and spawning patterns to local environmental conditions. Whether the basis for the distinct winter and spring spawning groups is genotypic or phenotypic has not been resolved, but the biological characteristics of the Bothwell's Creek populations merit further consideration.

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Recent Articles on FRB Investigations Published in Other Periodicals

Until 1970, reprints of these articles have been included in an annual volume of "Studies," composed of two parts. The final binding is the 1969 Studies, Part 2, which included No. 1355. These will no longer be bound and distributed. We will continue, however, to number and list all articles, which henceforth may be obtained in reprint form only from the author or originating FRB establishment. The boldface number following each title refers to the FRB establishment listed below.

(1) Fisheries Research Board of Canada
Biological Station
P.O. Drawer 100
Nanaimo, British Columbia

(2) Fisheries Research Board of Canada
Vancouver Laboratory
6640 N.W. Marine Drive
Vancouver 8, British Columbia

(3) Fisheries Research Board of Canada
Freshwater Institute
501 University Crescent
Winnipeg 19, Manitoba

(4) Fisheries Research Board of Canada
Arctic Biological Station
P.O. Box 400
Ste. Anne de Bellevue, Quebec

(5) Fisheries Research Board of Canada
Biological Station
St. Andrews, New Brunswick

(6) Fisheries Research Board of Canada
Marine Ecology Laboratory
Bedford Institute
P.O. Box 1006
Dartmouth, Nova Scotia

(7) Fisheries Research Board of Canada
Halifax Laboratory
P.O. Box 429
Halifax, Nova Scotia

(8) Fisheries Research Board of Canada
Biological Station
St. John's, Newfoundland

Studies No.

1398. LI, M. F., G. S. TRAXLER, AND L. M. LANGILLE. 1970. Toxic effect of elemental phosphorus on L-cells cultivated in suspension. Can. J. Zool. 48: 133–136. (7)

1399. PLATT, T. 1969. The concept of energy efficiency in primary production. Limnol. Oceanogr. 14: 653–659. (6)

1400. HURST, R. E. 1970. A gas chromatography collection syringe for volatile compounds. Chem. Ind. 1970: 90–92. (2)

1401. YAMAMOTO, M., AND K. L. MASSEY. 1969. Cyclic 3′,5′-nucleotide phosphodiesterase of fish (*Salmo gairdnerii*) brain. Comp. Biochem. Physiol. 30: 941–954. (2)

1402. YAMAMOTO, M. 1968. Fish muscle glycogen phosphorylase. Can. J. Biochem. 46: 423–432. (2)

1403. ANTIA, N. J., J. Y. CHENG, AND F. J. R. TAYLOR. 1969. The heterotrophic growth of a marine phostosynthetic cryptomonad (*Chroomonas salina*). Proc. Int. Seaweed Symp. 6: 17–29. (2)

1404. IDLER, D. R., B. TRUSCOTT, AND H. C. STEWART. 1968. Some distinctive aspects of steroidogenesis in fish. Excerpta Medica Int. Congr. Ser 184: 724–729. (7)

1405. BEARE-ROGERS, J. L., AND R. G. ACKMAN. 1969. Reaction of lipoxidase with polyenoic acids in marine oils. Lipids 4: 441–443. (7)

1406. PIPPY, J. H. C., AND G. M. HARE. 1969. Relationship of river pollution to bacterial infection in salmon (*Salmo salar*) and suckers (*Catostomus commersoni*). Trans. Amer. Fish. Soc. 98: 685–690. (5)

1407. WALDICHUK, M. 1968. Waste disposal in relation to the physical environment — oceanographic aspects. Syesis 1: 4–27. (1)

Guide to Authors

Details on typing and other items in preparing copy are given in a guide for typists. Also, a list of recommended spellings of about 100 words common in fisheries journals is available from the Office of the Editor.

EDITORIAL PRACTICES

An abstract must be provided in the same language as the text; authors are encouraged to provide both English and French versions.

The *Style Manual for Biological Journals* (2nd ed. American Institute of Biological Sciences, 2000 P St. N.W., Washington, D.C.) is, with few exceptions, used as the authority in matters of form. *Webster's Third New International Dictionary* is the standard for spelling and division of words in English; *le Grand Larousse Encyclopedique*, for those in French. Exceptions are made for manuscripts that follow the *Oxford English Dictionary* entirely. Titles of periodicals are abbreviated as in the *Biological Abstracts* list. References to literature are made by the name-and-year system. Manuscript reports are included in the list of references, but are identified both there and in the text by the letters "MS" before the year.

Notes are distinguished from *papers* on the basis of scope or completeness, rather than quality, of the research. They may be on problems with narrow, readily defined limits; on fishing gear or methods, or research techniques; on advances in knowledge arising as by-products of broader studies; on progress in projects that were discontinued before completion; or on preliminary findings that warrant publication before problems are resolved satisfactorily. Or they may be used to establish priority in publication. They may be organized with as much flexibility as papers except that they may have no centered headings. They should take no more than four pages in the *Journal*.

In general, *series titles* should be avoided.

Illustrations

Line drawings are preferably made with black india ink on white drawing or tracing paper or on coordinate paper with blue grids. All lines, symbols, letters, and numerals must be neat, and legible when the illustration is reduced to the appropriate size. Drawings should be grouped, when feasible, for economy of space. Originals must be submitted and should be less than 20 inches in the greater dimension for full-page illustrations, and proportionately smaller for those taking less than a page. Drawings must not be rolled for mailing.

Prints of photographs should be made on glossy paper with a wide range in tone. They should be cropped, squared, and mounted with rubber cement on white cardboard, with no space between those arranged in groups.

Proofs and Reprints

Galley proof is sent to the author, and he is responsible for detecting errors in printing as well as in content.

Fifty reprints, without covers, are supplied free. Additional copies needed must be ordered, on the form provided, when the galley proof is returned.

CHECKLIST ON MANUSCRIPTS

Attention to the following questions will expedite appraisal of manuscripts by referees and editors. The same questions are recommended to referees who review manuscripts for the *Journal*.

General

Is there new knowledge in the report worth publishing?

Are the findings, interpretations, and conclusions sound, and relevant to the purpose of the study?

If the manuscript is merely confirmatory, is it worth publishing?

Does the manuscript need to be rewritten before the work can be assessed?

Is the contribution placed in the proper perspective concerning the state of knowledge of the subject?

Is the manuscript too long?

Are all the tables and figures needed, and organized for economy of space?

Would any of the text be clearer if condensed?

Should some of the data be made available separately, in a manuscript report or at a data repository?

Should the manuscript be condensed as a Note?

Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Are summary statements given at the beginnings of sections and paragraphs, and are details in sections and paragraphs relevant to their topics?

Are there inconsistencies between tables or figures and the text, or within the text?

If the manuscript is from a thesis, does it retain characteristics common in these but not desirable in journals?

Parts of the Manuscript

Does the *title* encompass the content of the report?

Does the *abstract* give the essentials of the new knowledge?

Has the *introduction* all the important guideposts for orienting the reader?

Is review of the literature limited to defining the problem?

Are details of *materials and methods* limited to what scientists need in understanding the design of the study and in judging adequacy of the data obtained?

Are generalizations on the *results* adequate?

Are findings distinguished from inferences from them?

Is *discussion* limited to interpretation and significance of the findings?

If there are loopholes in interpretation, are they acknowledged? Should they be overcome?

Should other interpretations be recognized?

Guide for Typists Preparing Copy for

Journal of the Fisheries Research Board of Canada

Read the "Guide to Authors" in the current volume of the *Journal*. When in doubt about how to type or arrange details, follow *Journal* practice.

Type the manuscript on white bond paper, not flimsy, preferably size 8.5 × 11 inches (21.5 × 28 cm). If possible, use paper with the lines numbered in the margin. Leave all margins at least 1 inch (2.5 cm) wide. Use a nylon or carbon ribbon and keep the typewriter keys clean.

Double-space all parts of the manuscript, including tables, captions for illustrations, footnotes, and the reference list. Underline only material that is to be printed in italics. Indent the first line of each paragraph in the text and of each footnote.

In titles and headings, capitalize only the first word and proper names and adjectives. For small capitals, use small letters.

Indicate all text footnotes by superscript arabic numbers. Number them consecutively throughout the manuscript, starting with those on the title page. Place each footnote immediately below the reference to it, separating it from the text by horizontal lines.

On the first page type only the title, the authors' names and affiliations, related footnotes, and the running head (Appendix A). Type the abstract on a separate page (Appendix B). Number all pages, beginning with the title page and including those for tables and captions for illustrations.

Have the author check the finished manuscript, and send two copies (the ribbon copy and a carbon copy or a photocopy) to the Office of the Editor.

Tables Examine the current volume of the *Journal* to see how tables are set up (Appendix C). Note especially the following concerning *numbered tables*:

Type each table on a separate page, using paper without the lines numbered.

Number the tables with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, they are typed with the text, they seldom need horizontal lines, and the data are arranged in as few rows as possible (Appendix C).

Illustrations Send the original and two clear copies of each illustration with the manuscript. At the lower left of each figure and copy, give the author's name and the figure number. Also type each caption with its figure for identification. For photographs, type this information on a strip of paper and fasten it to the back of the print. Do not write or type on the print itself. Also, type the captions in succession on a separate page, headed "Captions for Illustrations." Put this page at the end of the manuscript.

Where each figure is first referred to, type in the margin "Fig. — near here."

Mathematical expressions, equations, and formulas Leave three spaces above and below lines that have much material in superscripts or subscripts, and above and below equations and formulas. In the various expressions, place each component in its correct position (Appendix D).

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter l and the numeral one, and between the capital letter O and the numeral zero. Make other explanatory notes similarly.

References Check the text to see that all references in the list are referred to. Type the reference list, double-spaced, in the style that is used in the *Journal* (Appendix E). Names of journals are abbreviated as in the *Biological Abstracts* list, but if an abbreviation is in doubt, type the name in full.

Spellings A list of about 100 words common in fisheries journals is given in Appendix F.

Appendices Appendices A to F are available on request from the Office of the Editor.

New

Fisheries Research Board of Canada Bulletin

Available Soon !

Freshwater Fishes of Northwestern Canada and Alaska

J. D. McPhail and C. C. Lindsey

"In the north exist many virgin freshwater environments unaffected by exploitation or pollution. These are the "baseline" that must be studied if we are to understand, and control the "stress" that we have already created wherever man is abundant."

The above quotation is taken from the Introduction of [illegible] upcoming Bulletin. This Bulletin is planned to serve several purposes and several audiences. The book

- deals with freshwater fishes in over 2 million square miles of one of the most sparsely populated regions of the globe
- contains complete descriptions and discussion of the 60 species of the region covered
- features 60 species illustrations, 50 distribution maps, 3 colour plates, additional 55 line drawings, 6 Keys, and many tables, figures, etc. not counted
- stresses the phenomena associated with postglacial dispersal, for there is probably no better region on earth for such investigation
- contains over 350 pages, $8.50

When [illegible] or inquiries concerning this book are received by the Fisheries Research Board, from the Queen's Printer for Canada, Ottawa, your local bookseller, or the Queen's Printer for Canada bookstore in

- Halifax: 1735 Barrington St.
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The above quotation is taken from the Introduction of FRB's newest upcoming Bulletin. This Bulletin is planned to serve several purposes and several audiences. The book

- deals with freshwater fishes in over 2 million square miles of one of the most sparsely populated regions of the globe
- contains complete descriptions and discussions of the 60 species of the region covered
- features 60 species illustrations, 59 distribution maps, 5 colour illustrations, 55 line drawings, 6 Keys, end-sheet maps showing the area covered
- stresses the phenomena associated with postglacial dispersal, for there is probably no better region on earth for their investigation
- contains over 350 pages, $8.50

When available (watch for further announcement on this page), this Bulletin will be obtainable from the Queen's Printer for Canada, Ottawa, your local bookseller, or the following Queen's Printer for Canada bookshops:

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Queen's Printer for Canada, Ottawa, 1970